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QUANTITATIVE EXPERIMENTS ON THE LIBERATION OF EPINEPHRIN FROM THE ADRENALS AFTER SECTION OF THEIR NERVES, WITH SPECIAL REFERENCE TO THE QUESTION OF THE INDISPENSABILITY OF EPINEPHRIN FOR THE ORGANISM

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It is known that after section of certain nerves the rate of the spontaneous liberation of epinephrin is greatly reduced. This is very easy to demonstrate in the cat in acute experiments by the method previously described by us (1) (collection of adrenal vein blood in a cava pocket, and the action of the blood when released in eliciting dilatation of the pupil and retraction of the nictitating membrane in the eye previously prepared by Meltzer's procedure, so as to react with great sensitiveness to epinephrin). After section of the fibers coming to the semilunar ganglion, including the splanchnics, or after section of the two sympathetic trunks, including the splanchnics, in the thorax near to the diaphragm, the eye reactions are in general no longer obtained, unless with considerably longer periods of collection of blood than were sufficient to elicit them strongly before the nerve section. The rise of blood pressure associated with the release of blood containing epinephrin is likewise missing after the nerves have been severed. In survival experiments also it was shown that when the right adrenal was excised and the fibers coming to the left semilunar ganglion cut the eye and blood pressure reactions could no longer be detected. Since cats survive this operation indefinitely, and so far as can be seen after recovery from the operation in the same health and vigor as normal animals, the experiments obviously have a bearing

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on the question whether epinephrin is indispensable to the organism. It therefore becomes important to determine the magnitude of the residual liberation of epinephrin, if any, from the denervated gland.

It is clear that for this purpose everything depends upon the delicacy of the reactions relied upon for the detection and assay of epinephrin. We made assays with the denervated eye and blood pressure reactions by determining the amounts of adrenalin which must be injected to give approximately the same reaction as the blood released from the cava pocket. When these reactions were found to be absent after the nerve section the amount of adrenalin was determined which could just be detected in this way with certainty. An upper limit was thus fixed to the possible amount of the residual epinephrin. In using these eye reactions, however, the epinephrin-containing blood is necessarily greatly diluted before it reaches the reacting structures. These observations have accordingly been supplemented by a series of experiments in which the blood was drawn off from the cava pocket and tested directly on rabbit intestine and uterus segments. The normal output of epinephrin, under the experimental conditions, as assayed by the eye (and blood pressure) reactions in eight cats was found to range from 0.0008 to 0.0028 mgm. per animal per minute (average, 0.0017 mgm.). The output per minute per kilo of body weight ranged from 0.0003 to 0.0008 mgm. (average 0.0006 mgm.). The data of six of these cats have already been published in the previous paper, on spontaneous liberation of epinephrin.[1] The observations on the remaining two are given in table 1.

In ten normal cats taken at random from the stock the assays of the spontaneously liberated epinephrin by direct application of the adrenal blood to rabbit intestine and uterus segments

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[1] Journ. Pharm. Exp. Ther., 1916, viii, table 8, p. 500. The opportunity may be taken to correct an error in table 8. The concentrations of epinephrin given in the last column of the table should be deleted.

TABLE 1

NUMBER OF CAT	BODY WEIGHT	WEIGHT OF ADRENALS	DURATION OF POCKET	AMOUNT OF ADRENALIN INJECTED	EPINEPHRIN	
					Per animal per minute	Per kilo per minute
	kgm.	grams	seconds		mgm.	mgm.
85	3.16	0.422	60	0.5 cc. 1 : 500,000	0.001	0.0003
			30	+0.5 cc. 1 : 1,000,000	0.001+	0.0003+
			120	+0.5 cc. 1 : 250,000	0.001+	0.0003+
86	2.875	0.680	60	−0.5 cc. 1 : 250,000	0.002−	0.0007−
			15	+0.5 cc. 1 : 1,000,000	0.002+	0.0007+
			60	0.5 cc. 1 : 250.000	0.002	0.0007
			15	+0.5 cc. 1 : 1,000,000	0.002+	0.0007+

In cat 85, the renal, coeliac and mesenteric arteries and the abdominal aorta were tied; in cat 86, only the renals and abdominal aorta. The sign + or − before the amount of adrenalin injected, indicates that a little more or a little less would have been necessary to give a reaction equal to that produced by the blood in the corresponding cava pocket. The adrenalin solutions were made up in 0.9 per cent sodium chloride solution and were washed in with sodium chloride solution from a small burette. The stock adrenalin was always assayed.

gave an output per minute per animal ranging from 0.0004 to 0.001 mgm. (average, 0.00065 mgm.); and an output per kilo of animal per minute, ranging from 0.0002 to 0.00045 mgm. (average, 0.00025 mgm). The details are given in table 2. As will be seen, the results in the different animals vary surprisingly little. The same is true for the different animals examined by the eye reactions. It is, therefore, a striking fact that on the average only about half as much epinephrin is estimated by the rabbit segments as by the eye reactions. It is quite impossible to explain this difference as due to accidental variations in the rate of output in the animals of the two series. It must, therefore, be concluded that some of the epinephrin is lost when the adrenal vein blood is drawn, in the interval which necessarily elapses, and under the manipulations which the blood necessarily undergoes before it is applied to the segments.

There is another reason for the deficiency in the assays on the drawn blood, although this has been eliminated as far as possible in the observations comprised in the Table by choosing for the assay only samples collected while the blood flow was good. We have found abundant evidence that no matter how slow the flow through the adre-

TABLE 2

NUMBER OF CAT	BODY WEIGHT	WEIGHT OF ADRENALS	ADRENAL BLOOD SAMPLE	BLOOD FLOW				EPINEPHRIN CONCENTRATION	EPINEPHRIN	
				Grams	In minutes and seconds		Gms. per minute		Per animal per minute	Per kilo per minute
					minutes	seconds				
	kgm.	grams							mgm.	mgm.
64	2.425	0.390	1	0.9		20 ·	2.7			
			2	1.9	1	30	1.3	1:2,000,000	0.0006	0.00025
			3	2.6	2	10	1.3	1:2,000,000	0.0006	0.00025
			4	4.3	3	20	1.3	1:2,000,000	0.0006	0.00025
			5	3.3	3		1.1			
			6	4.0	4		1.0			
63	3.41	0.550	1	2.0		55	2.2			
			2	2.9	1	15	2.3	1:2,000,000	0.001	0.0003
			3	3.1	1	20	2.4			
			4	3.3	1	45	1.9	1:2,000,000	0.001	0.0003
			5	4.0	2	5	2.0	1:2,000,000	0.001	0.0003
			6	3.2	2		1.6			
			7	2.0	1		2.0			
			8.	6.5	4	25	1.5			
65	2.035	0.338	1	2.5	1		2.5			
			2	4.5	2		2.2	1:2,500,000	0.0009	0.00045
			3	3.6	2	45	1.3	1:1,500,000	0.0009	0.00045
			4	2.5	3	30	0.7			
			5	1.7	4		0.4	1:1,000,000	0.0004	0.0002
67	2.31	0.440	1	2.0		20	6.0			
			2	10.2	2		5.0	1:13,000,000	0.0004	0.0002
81	1.98	0.396	1	3.6	1	20	2.8			
			2	9.9	5		2.0			
			3	6.8	6		1.13	1:2,200,000	0.0005	0.00025
			4	3.0	4	30	0.66			
82	2.66	0.300	1	5.3	2	30	2.1			
			2	8.3	4		2.1	1:4,500,000	0.0005	0.0002
			3	9.0	5		1.8			
			4	8.8	·6		1.5			
			5	6.9	7		1.0	1:2,300,000	0.00045	0.0002
			6	4.5	8		0.56			
83	3.03	0.360	1	4.7	2		2.3			
			2	7.8	4		1.9	1:3,200,000	0.0006	0.0002
			3	10.1	6		1.7			
			4	9.8	8		1.2	1:2,000,000	0.0006	0.0002
			5	3.5	9		0.4			

TABLE 2—Continued.

NUMBER OF CAT	BODY WEIGHT	WEIGHT OF ADRENALS	ADRENAL BLOOD SAMPLE	BLOOD FLOW				EPINEPHRIN CONCENTRATION	EPINEPHRIN	
				Grams	In minutes and seconds		Gms. per minute		Per animal per minute	per kilo per minute
	kgm.	grams			minutes	seconds			mgm.	mgm.
84	2.975	0.580	1	5.2	2		2.6			
			2	9.3	4		2.3	1:4,000,000	0.0006	0.0002
			3	11.6	5		2.3	1:4,000,000	0.0006	0.0002
			4	10.6	5		2.1			
			5	11.1	6		1.8	1:2,700,000	0.0007	0.00025
			6	5.7	10		0.5			
85	3.16	0.422	1	3.6	2		1.8			
			2	5.2	4		1.30	1:1,700,000	0.0008	0.00025
			3	7.3	10		0.73	1:1,000,000	0.0007	0.0002
			4	1.6	8	30	0.2			
86	2.875	0.680	1	3.8	4		0.95			
			2	4.7	8		0.6	1:1,500,000	0.0004	0.00015
			3	1.6	4		0.4			

In all the cats except 84 and 86 all the arteries (renal, coeliac, mesenteric and abdominal aorta) were tied. In 84 and 86 the coeliac and mesenteric arteries were not tied. All the cats except 65 were anesthetized with urethane. Cat 65 was rendered insensitive by increased intracranial pressure. In cat 64, in addition to urethane anesthesia, the intracranial pressure was increased. In cat 67 the unusually low concentration of epinephrin was associated with an exceptionally large blood flow, but no unligated small vein could be found.

nals may be, the concentration of epinephrin in the blood of the adrenal veins (in cats) cannot rise beyond a certain maximum (not very far from 1:1,000,000 as assayed by rabbit segments). When this maximum has once been reached further diminution of the rate of blood flow necessarily leads to a diminished output per minute. When the blood flow is free, and the concentration well below this limiting value, it can be proved that within a wide range the rate of flow and the concentration of epinephrin vary inversely, the rate of liberation of the epinephrin per minute remaining constant. Since when blood is drawn off from a cannula in the cava pocket the rate of flow is nearly always diminished to some extent, as compared with the rate of flow in the eye observations, which do not entail the drawing off of blood, the output as estimated from the epinephrin content in the drawn blood may easily be below the true value unless samples collected with a sufficient blood flow are used for the assay.

The fact that the earlier adrenal specimens usually have a smaller concentration than the later ones, associated with gradual decline in the rate of blood flow, indicates that the cause of the smaller output per minute estimated on intestine and uterus segments in shed adrenal blood, as compared with the output determined by the eye and blood pressure reactions without drawing blood, is not the loss of epinephrin withdrawn from the circulation. For the effect of epinephrin already circulating in the blood, if any appreciable amount at all were present in the general blood, ought to be most marked in the first specimens.

In two experiments (cats 85 and 86, tables 1 and 2) the epinephrin output was estimated both by the eye reactions and in the drawn adrenal blood by the rabbit segments. In cat 85, in which the output, as determined by the eye reactions, was of fair average magnitude, 0.001 mgm. per minute, the adrenal blood assays yielded values not very far inferior, namely, 0.0008 and 0.0007 mgm. in two samples. The blood flows, while the adrenal samples were being collected, were fairly good; although probably below the flow while the eye reactions were being observed. In cat 86, on the other hand, in which the output as determined by the eye reactions, was very good, 0.002 mgm. per minute, the deficiency in the assays of the adrenal blood on the intestine segments was very considerable; and the blood flow during collection of the samples was rather poor—probably much less than during the testing by the eye reactions. It is obvious, then, that if the question whether the output in an animal is below the normal range or not is being considered, it will not do to compare assays made by the one method with assays made by the other; at any rate, unless the differences are considerable. Fortunately for our purpose, the reduction in the epinephrin output by section of the adrenal nerves is so enormous that it makes not the slightest difference whether we compare the residual liberation with normal values obtained by the eye (and blood pressure) reactions or by the rabbit segments.

Before proceeding to the consideration of the experiments, it may be well to point out that as regards the question of a residual secretion of epinephrin by the adrenals after all the secretory

nerve fibers have been cut, as far as is possible, negative results are much more important than positive ones, always provided that the negative results have been obtained on test objects of the highest degree of sensitiveness. For while the course of the bulk of the secretory fibers has been ascertained, and they can be easily severed, it is impossible to be sure in any given operation that some have not escaped.[2]

Apart from the surgical risks, it is undesirable in acute experiments to go around the glands with an instrument, since not only is the lymph flow thus necessarily interfered with, but the chance of causing liberation of epinephrin by massage is great. Even then it would be impossible to be sure that some of the fibers in question did not pass in along the blood vessels. In survival experiments, although the effect of massage would introduce no error, the glands would be liable to be affected by adhesions as well as by interference with the lymphatics, if the tissues in their immediate neighborhood were freely divided.

Another reason why experiments yielding well determined negative results with very sensitive test objects must for our problem carry greater weight than experiments in which small quantities of epinephrin are still found in the adrenal blood, even after extensive nerve sections, is that the necessary manipulations in obtaining the blood, the exposure of the glands and the possible disturbance of the circulation in them, might cause a small amount of the epinephrin, known to be present in the glands in not less than normal amount, to escape into the blood. This remark is not intended to imply that when due precautions are taken, it is difficult to avoid the liberation of epinephrin in appreciable amounts in the absence of the secretory nerves. On the contrary, we have never had any reason to suspect that when epinephrin was found in any of the adrenal blood samples, except perhaps in the first small sample, it had not been liberated in the normal way. Even in the preliminary sample, always collected apart with the object of allowing any epinephrin already present in the adrenal capillaries or in the cava pocket itself to be washed out, and which not quite accurately we fell

[2] Elliott, Journ. Physiol., 1912, xliv, 374, has shown that section of the preganglionic fibers of the semilunar ganglion, including the splanchnics, prevents exhaustion of the store of epinephrin under the influence of various conditions. He made no experiments on the question whether after this operation the liberation of epinephrin is suppressed, and assumes, indeed, that "ultimately" it is resumed, since "the decentralized gland suffices to keep the animal alive."

into the habit of speaking of as the "manipulation sample," we generally found no more epinephrin than in succeeding samples; sometimes, indeed, less, on account of a certain amount of ordinary cava blood being retained in the pocket as it was being closed off.

It may be further remarked that as between the acute and the survival experiments greater weight should be attached to the latter, when the absolute amount of any residual epinephrin liberation is in question. The acute experiments were made mainly to get a general idea of the immediate diminution in the rate of liberation when the nerves were severed. It was desired also to see the effect of piece-meal sectioning of the nerve supply. It was recognized, however, that although a marked immediate diminution in the output was to be looked for, the full effect might not be expressed in the epinephrin content of the adrenal vein blood collected during a short experiment. Apart altogether from the possibility that some irritation of the cut nerves might persist, there was the possibility that epinephrin already in the adrenal capillaries might continue to be washed out in small quantities for some time; or even that a certain amount of epinephrin not yet actually in the blood might be in such a situation, or perhaps in such a combination, at the moment of section of the nerves that it could migrate into the blood stream in the next few minutes without the influence of nerves.

Finally, it must not be forgotten that minor differences in the innervation may occur in different individuals, so that a nerve section which in one cat brings the epinephrin output below the threshold of detectability, say by the eye reactions, need not do so in the case of another cat. We have given illustrations of this in a previous paper (2). In this question there is involved, of course, not merely the possibility of slight anatomical variations; but also the known variability in sensitiveness of the test objects, and the degree of activity of the gland at the moment of section of the nerves. If a relatively large liberation were going on at that moment it is conceivable that a small number of fibers remaining uncut might sustain a detectable output, whereas with a gland less responsive to its innervation the same fraction of the total number of fibers might be unable to do so. The possibility must also be taken into account that a small strand might be overlooked in one

operation which was cut in another, a risk, of course, present especially in the survival experiments where aseptic operations had to be performed, although the large number of times the main operation had been previously done greatly reduced this risk. The remarkable agreement in the general result of the survival experiments shows that any variation due to this factor cannot have been great.

<div align="center">ACUTE EXPERIMENTS</div>

Three such experiments were made on cats and two on dogs. The nerve supply of the adrenals, at least the path of the fibers whose section protects the epinephrin store, and of those whose stimulation increases the epinephrin liberation, has been more completely studied in the cat than in the dog. The experiments on cats are therefore more valuable for the determination of the question how far a complete section of the nerves affects the rate of liberation.

Experiment 1. Condensed protocol. Cat. Weight, 2.7 kgm.
10.30 a.m.   3 grams urethane.
12.05 p.m.   Collected specimen of jugular blood; tied off cava pocket, also renal, coeliac and mesenteric arteries.
12.55 p.m.   Began collection of adrenal samples as follows:

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE
	grams	*minutes*	*seconds*	*grams*
1	3.0	2	43	1.1
2	2.5	3	25	0.73
3	2.7	3	25	0.79
4	1.8	7		0.26

Both sympathetics in the thorax were cut at the end of collection of the second blood specimen just at the origin of the splanchnics below the thirteenth rib. The nerves had been isolated on loose ligatures before collection of adrenal blood was begun. Combined weight of adrenals, 0.392 gram.

It is evident from figure 1, that the first adrenal blood specimen (observation 3), collected before section of the sympathetics, contained a much greater concentration of epinephrin than the fourth specimen (observation 7), collected after the section.

The same thing was shown by comparison of the second specimen (collected before the nerve section) with the third specimen, collected immediately after the section. As this experiment was intended to be mainly a preliminary qualitative one, no very exact assay of the epinephrin concentration was made on the intestine segments. However, it was found that even the fourth sample, which on account of the slow flow would normally have been rich in epinephrin, had a concentration much less

Fig. 1. Intestine Tracings.   Bloods from Cat Anesthetized with Urethane

At *2* Ringer was replaced by indifferent (jugular) blood; and this at *3* by the first adrenal blood specimen (collected before section of sympathetics).   At *6* Ringer was replaced by jugular blood and this at *7* by the fourth adrenal specimen (collected after section of the sympathetics).   All the bloods were diluted with an equal volume of Ringer.   As in all the figures, time is marked in half minutes.   (Reduced to one-half.)

than 1:24,000,000, and that the rate of liberation of epinephrin per minute after section of the nerves must have been much less than one-thirtieth of its original amount.

Experiment 1 accordingly shows that section of the two sympathetic trunks at the origin of the major splanchnics reduced to a small fraction of its previous amount the output of epinephrin, which, however, was not abolished.

Experiment 2. Condensed protocol. Cat. Weight, 2.64 kgm. Left superior cervical ganglion excised eighteen days before experiment. Condition excellent.

11.00 a.m.   4 grams urethane.

12.45 p.m.   Obtained jugular blood. From 1.00 p.m. to 1.25 p.m., completed cava pocket, tying abdominal aorta below renal arteries.

TIME		DURATION OF POCKET		PUPIL DILATATION AFTER	NICTITATING MEMBRANE
		minutes	seconds	seconds	seconds
1.30	Pocket experiment.. Nerves coming to both semilunar ganglia cut	1		Very good, 6.4	Very good, 6.4
1.50	Pocket experiment..	1	10	No	No
1.53	Pocket experiment..	2	10	No	Slight, 11
2.04	Both semilunar ganglia excised.......				
2.10	Pocket experiment..	4		Small, 10.2	Small, 10.2
2.20	Lumbar sympathetic cut below diaphragm..........				
2.40	Pocket experiment..	4		Small, 18.6	Small
2.51	Cut sympathetic trunks in thorax at origin of splanchnics.............				
2.55	Pocket experiment..	4	30	No	Slight
3.10	Pocket experiment..	4	30	Very slight 20.4	Very slight, 20.4

3.13 p.m.   Began collection of adrenal blood from pocket. Flow very slow. During fifteen minutes only about 0.5 cc. collected. Then clotting occurred and by removing the clots from time to time another 0.5 cc. was obtained.

This experiment shows that after section of the preganglionic fibers coming to the semilunar ganglion on both sides, slight eye reactions for epinephrin were still obtained. But even with a longer time of collection of blood in the cava pocket the reactions were much reduced. That is to say, the rate of liberation of epinephrin was markedly diminished. After excision of both semilunar ganglia and consequent division of any strands

which might have escaped section before, the time of collection had to be still further increased to give a noticeable reaction. Section of the lumbar sympathetic chain, one ganglion below the diaphragm, caused no further alteration in the reaction. When both sympathetics were now divided in the thorax, a very slight eye reaction was still elicited with a long period of collection.
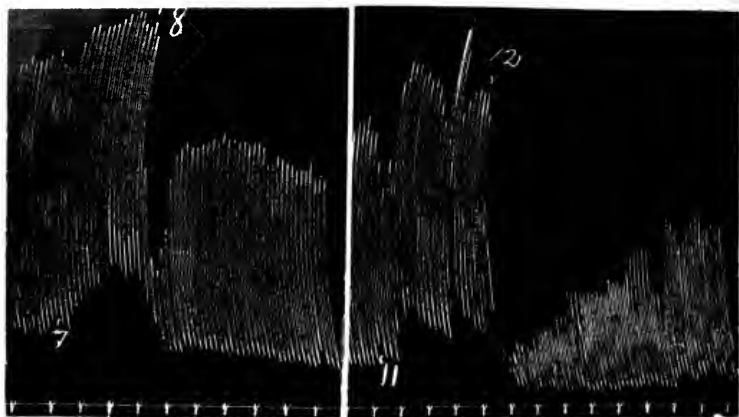
FIG. 2. INTESTINE TRACINGS. BLOODS FROM CAT ANESTHETIZED WITH URETHANE

At *7* Ringer was replaced by jugular blood, and this at *8* by an adrenal blood specimen collected with very slow blood flow after section of nerves to both semi-lunar ganglia and section of sympathetics in thorax. At *11* Ringer was replaced by jugular blood, and this at *12* by blood containing 1:1,600,000 added adrenalin. All the bloods were diluted with three volumes Ringer before application to the segments. (Reduced to two-thirds.)

A sample of blood obtained from the cava pocket after section of all these nerves contained much less than 1 : 1,660,000 of epinephrin (fig. 2). The adrenalin assay on intestine segments showed further that the concentration in this blood was less than 1 : 3,300,000, but more than 1 : 5,000,000. The flow was very slow when the blood was being collected, doubtless much slower than when the eye observations were being made. In the cat with intact nerves, a considerably greater concentration would be expected with such a small flow. Even if none of the

epinephrin in the blood was due to "manipulation," the rate of liberation must have been reduced by the nerve section, probably to one-fiftieth of its normal amount, as estimated on intestine segments. The rate of liberation per minute does not vary much in the course of an experiment, even when the blood flow diminishes, so long as the concentration is well below the possible maximum, as in this case, increased concentration compensating for lessened flow.

Experiment 3. Condensed protocol. Cat. Weight, 1.41 kgm. Left superior cervical ganglion excised three weeks before the experiment. Condition excellent.

11.20 a.m.   3 grams urethane.

12.45 p.m.   Inserted tracheal cannula and made cava pocket, tying off renal arteries and abdominal aorta below renals. The left nictitating was retracted, and nictitating reactions could not be used.

TIME		DURATION OF POCKET		PUPIL DILATATION AFTER
		minutes	*seconds*	*seconds*
1.20	Pocket experiment...............	1		Good 6.8
1.22	Pocket experiment with left adrenal vein clipped.............	2		Good 7.4
1.30	Right sympathetic in thorax cut at origin of splanchnic			
1.37	Pocket experiment; left adrenal vein clipped...................	2		No
1.42	Left sympathetic cut in thorax one ganglion above splanchnic origin.			
1.43	Pocket experiment...............	2		No
1.46	Pocket experiment...............	3	10	No

Inserted cannula into right iliac vein, and collected adrenal blood: First specimen, 1 gram in 16.5 minutes (0.06 gram per minute); second specimen, 1.4 grams in 35 minutes (0.04 gram per minute). The blood flow was very slow. While these specimens were being collected, a specimen of jugular blood was obtained. After collection of the adrenal blood a specimen of arterial blood was got from the abdominal aorta, with the cava pocket still closed. Combined weight of adrenals, 0.31 gram.

In this experiment, the eye reaction could no longer be elicited after section of both sympathetic trunks, including the major and minor splanchnics in the thorax. Adrenal vein blood collected after the nerves had been severed showed a concentration of epinephrin by rabbit intestine segment tests of not more than 1 : 1,500,000. As the flow was extremely slow (0.05 gram per minute, or one-twentieth of an ordinary flow), the relatively high rate of concentration is what would be expected so long as any appreciable amount of epinephrin was being given off. The rate of liberation, however, after the nerve section was only one-tenth to one-twentieth of the normal rate (about 0.00003 mgm. per minute, or 0.00002 mgm. per kilo per minute) (see table 2). The epinephrin assay in this experiment was not entirely satisfactory, because there was reason to believe from the uterus tests that a part of the inhibition of the intestine segment was due to some other substance than epinephrin. It is known that sometimes in asphyxia such a substance appears in venous blood, and even in arterial blood; and its appearance in this experiment might have been associated with the very small blood flow at the time of collection of the adrenal specimens. If this was the case the reduction in the rate of epinephrin output must have been even greater than the calculated reduction. On the other hand, since as already pointed out there is a limit of epinephrin concentration not very far above that found in the adrenal blood in this case, and which is never exceeded in our experience no matter how small the rate of blood flow may be, the question may fairly be asked whether with extremely small flows it is legitimate to assume that the gland is liberating as much epinephrin in response to the residual innervation as it would have done had the flow been larger. As regards our main conclusion, however, this objection has no weight. For not only were the pupil reactions abolished in this experiment by the nerve sections at a time when the blood flow was good, although quite strongly elicited before the nerves were cut; but what is more important exceedingly small blood flows were found associated in some of the survival experiments either with remarkably low concentrations of epinephrin, as determined by the segment

tests, or with a complete absence of epinephrin in detectable amount.

Although there was no reason to assume that the small residual output of epinephrin in these acute experiments was due to anything else than some small uncut remnant of nerve fibers, two acute experiments were made on dogs in order to take advantage of the larger blood flow through the adrenals, which might be expected to wash out more thoroughly any epinephrin already liberated before the various nerve sections. As already stated, however, this advantage was counterbalanced by the less precise knowledge of the nerve paths in the dog; and although the nerve sections practiced reduced the rate of liberation of epinephrin to a mere fraction of its initial amount, a substantial residual output remained.

Experiment 4. Condensed protocol. Dog. Weight, 7.75 kgm. Anesthetized with ether.

9.10 a.m.   Tracheal cannula inserted. Specimen of jugular blood obtained. Right adrenal gland excised. Cava pocket made with a cannula in each iliac vein. The following specimens of adrenal blood were obtained through the right iliac cannula: First adrenal specimen, 5 grams in 1 minute, 30 seconds (3.4 grams per minute); second specimen, 7.2 grams in 2 minutes, 16 seconds (3.2 grams per minute).

10.15 a.m.   Cut left major and minor splanchnics in abdomen; dissected around left adrenal, cutting nerve connections; excised three lumbar sympathetic ganglia, including chain from diaphragm downward. Then collected through left iliac cannula the following adrenal blood specimens: Third adrenal specimen, 4.3 grams in 5 minutes, 10 seconds (0.8 gram per minute); fourth specimen, 3.0 grams in 6 minutes, 30 seconds (0.5 gram per minute). Pulled out clot from cannula and flow improved. Collected the following specimens: Fifth adrenal specimen, 5.0 grams in 4 minutes, 10 seconds (1.2 grams per minute); sixth specimen (6.5 grams in 6 minutes (1.1 grams per minute); seventh specimen, 4.5 grams in 4 minutes (1.1 grams per minute); eighth

specimen, 3.5 grams in 4 minutes, 30 seconds (0.9 gram per minute). During the latter part of the collection of the eighth specimen, the left sympathetic was cut in the thorax at origin of the splanchnics. The following adrenal blood specimens were then collected: Ninth specimen, 4.8 grams in 4 minutes, 30 seconds (1.1 grams per minute); tenth specimen, 3.8 grams in 8 minutes (0.5 gram per minute). Arterial blood was now obtained from the abdominal aorta, the cava pocket being still closed off.

The rabbit segment tests showed that the concentration of epinephrin in the adrenal blood collected before the nerve
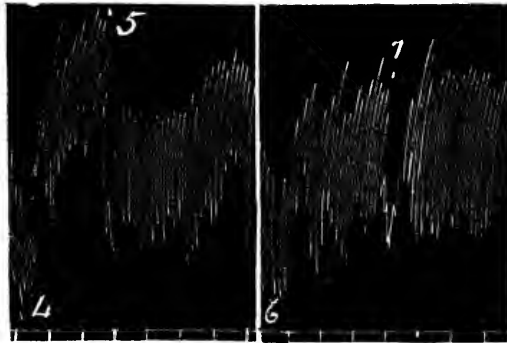
FIG. 3. INTESTINE TRACINGS. BLOODS FROM DOG ANESTHETIZED WITH ETHER, AND WITH RIGHT ADRENAL EXCISED

At *4* Ringer was replaced by indifferent (jugular) blood, and this at *5* by the second adrenal specimen (collected before section of nerves). At *6* Ringer was replaced by jugular blood, and this at *7* by the sixth adrenal specimen (collected after denervation of the left adrenal). The bloods were diluted with an equal volume of Ringer. (Reduced to two-thirds.)

sections was decidedly greater than in the blood collected after the nerve sections (figs. 3 to 5). Some of the tracings taken for assaying the amount of epinephrin in adrenal samples before and after division of the nerves are reproduced in figures 4 and 5. The concentration in the second sample (fig. 3, observation 5) is less than 1:5,000,000, much greater than 1:20,000,000, and not far from 1:7,000,000 (fig. 4, observations 9, 11, 15). A
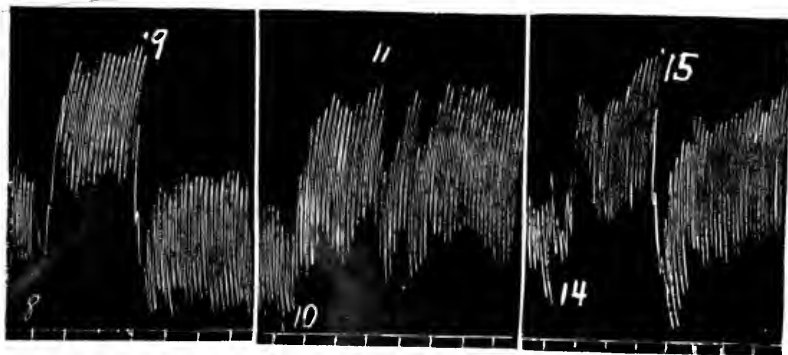
FIG. 4. INTESTINE TRACINGS.   BLOODS FROM SAME DOG AS IN FIGURE 3

At *8* Ringer was replaced by jugular blood diluted with an equal volume of Ringer, and this at *9* by jugular blood made up to 1 : 5,000,000 adrenalin, and then diluted with an equal volume of Ringer.   At *10* and *14* Ringer was replaced by jugular blood diluted with an equal volume of Ringer and this at *11* and *15*, respectively, by jugular blood made up to 1 : 20,000,000 adrenalin, and to 1 : 7,000,-000 adrenalin, and then diluted with an equal volume of Ringer.   (Reduced to two-thirds.)
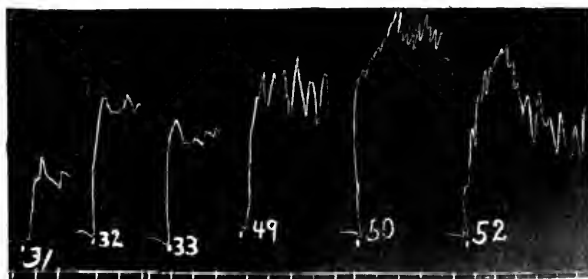
FIG. 5. UTERUS TRACINGS.   BLOODS FROM SAME DOG AS USED IN FIGURES 3 AND 4

At *31* Ringer was replaced by indifferent (arterial) blood, at *32* by the second adrenal specimen (collected before section of nerves); at *33* by the sixth adrenal specimen (collected after section of nerves).   These bloods were undiluted. At *49* Ringer was replaced by the tenth adrenal specimen (collected after section of the nerves).   The blood was diluted with an equal volume of Ringer.   At *50* Ringer was replaced by jugular blood to which was added adrenalin to make up 1 : 10,000,000, and the blood then diluted with an equal volume of Ringer.   At *52* Ringer was replaced by jugular blood to which was added adrenalin to make up 1 : 20,000,000, the blood being then diluted with an equal volume of Ringer. (Reduced to one-half.)

test made with the second adrenal sample immediately after observation 15 gave a curve more nearly resembling that of observation 15, but showing slightly smaller inhibition of the intestine. The sixth adrenal sample contained obviously much less than 1: 7,000,000 epinephrin. Another observation on the intestine, not reproduced, showed that the concentration in this sample was greatly inferior to 1: 10,000,000. Observation 11 (fig. 4) indicates that the concentration in the sixth sample was not far from 1: 20,000,000. This was confirmed on the uterus. Observation 49 (fig. 5), indicates that the effect of the tenth adrenal sample was much less than 1: 10,000,000 adrenalin (observation 50), and not very different from 1: 20,000,000 (observation 52). On the intestine it was shown, on tracings not reproduced, that the tenth sample caused a slightly greater inhibition than the sixth sample, and this was corroborated by uterus tracings. The rate of flow when the second sample was being collected was about three times as great as during collection of the sixth sample. The concentration of the sixth sample being approximately one-third that of the second, the rate of liberation of epinephrin per minute must have been reduced at least to one-ninth of its original value in consequence of the nerve sections. It must be remembered that the minute-output of epinephrin is that due to one adrenal only. The concentration, however, would not be affected by this circumstance, since one adrenal having been excised, the blood flowing into the cava pocket was of course correspondingly reduced.

It may appear somewhat surprising that in spite of the drastic procedure adopted to sever the secretory nerves of the left adrenal, the rate of liberation of epinephrin was still a substantial fraction of its initial value. Subsequent section of the sympathetic trunk in the thorax, as was to be expected, did not alter this fraction perceptibly. It is quite possible, as suggested before, that the attempt to cut fibers close to the adrenal caused such disturbance in the gland by altering the circulation, and perhaps in other ways, that some epinephrin continued to be discharged during the remainder of the experiment independently of the central innervation.

In the next experiment disturbance of the adrenal was avoided by dividing the sympathetics in the thorax just below the twelfth rib. This operation at once reduced the rate of output of epinephrin to not more at most than one-twelfth of its initial value. That the residual liberation was sustained in the same way as the normal initial output, that is, through nerves which had escaped section, is suggested by the fact that after section of the nerves successive specimens of adrenal vein blood (and serum) showed the same progressive increase in the concentration of epinephrin as before the section. The reduced rate of liberation per minute therefore remained practically independent of the rate of blood flow, just as the original rate did before the nerve section, the concentration varying approximately in the inverse ratio of the blood flow.

Experiment 5. Condensed protocol. Dog. Weight, 18.35 kgm. Anesthetized with ether.

10.40 a.m.   Tracheal and jugular cannulae inserted.

10.50 a.m.   Obtained jugular blood. Cava pocket made, and cannula inserted in the right iliac vein. The following specimens of adrenal blood were collected: First specimen, 36.2 grams (time record lost); second specimen, 30 grams in 1 minute, 10 seconds (26.1 grams per minute); third specimen, 26.0 grams, in 1 minute, 20 seconds (19.5 grams per minute). Cut both sympathetics in thorax one ganglion above origin of splanchnic. Collected the following specimens of adrenal blood: Fourth specimen and fifth specimen not satisfactorily collected; sixth specimen, 22 grams in 1 minute, 37 seconds (13.6 grams per minute); seventh specimen, 33 grams in 2 minutes, 25 seconds (13.2 grams per minute). Combined weight of adrenals, 1.480 grams. The bloods were centrifuged and the sera tested on rabbit segments.

Of the numerous observations made to assay the epinephrin content of the sera only four are reproduced (figs. 6 and 7). Figure 6 shows that the third adrenal sample taken just before section of the nerves had a much greater concentration of epinephrin than the sixth sample, collected after the nerve section.

FIG. 6. BLOODS FROM DOG ANESTHETIZED WITH ETHER

At *5*, Ringer was replaced by the serum of jugular blood, and this at *6* by the serum of the sixth adrenal blood specimen (collected after section of the sympathetics in the thorax).   At *15* Ringer was replaced by the serum of jugular blood, and this at *16* by the serum of the third adrenal specimen (collected before section of the sympathetics).   All the sera were diluted with an equal volume of Ringer. (Reduced to one-half.)
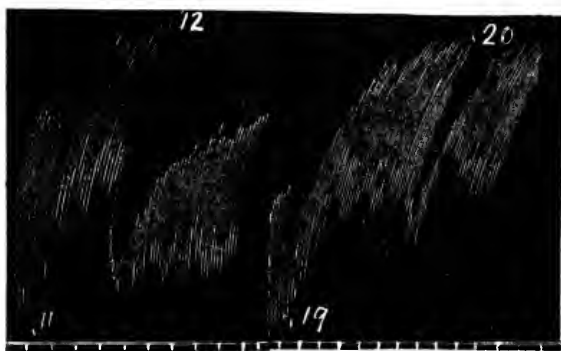
FIG. 7. INTESTINE TRACINGS.   BLOODS FROM SAME DOG AS USED IN FIGURE 6

At *11* Ringer was replaced by serum of jugular blood, and this at *12* by serum of jugular blood to which had been added adrenalin to make up 1 : 3,000,000.   At *19* Ringer was replaced by serum of jugular blood, and this at *20* by serum of jugular blood to which had been added adrenalin to make up 1 : 20,000,000.   All the sera were diluted with an equal volume of Ringer.   The concentrations of adrenalin given are the concentrations in the serum before this dilution.   (Reduced to one-half.)

Observation 12 (fig. 7) indicates that the concentration in the serum of the third sample was decidedly greater than 1 : 3,000,000. The concentration in the serum of the sixth sample was somewhat greater than 1 : 20,000,000 (observation 20). Other observations with various concentrations of adrenalin confirmed the conclusion that the concentration in the sixth sample was not far from one-eighth of that in the third sample. The rate of flow of the third sample was approximately in the ratio of 3 : 2 to that of the sixth. Therefore, the rate of liberation of epinephrin per minute was diminished to about one-twelfth by the nerve section.

The results of the acute experiments can be summed up in a sentence. In none of them have we failed to find in the adrenal vein blood after section of the nerves a sufficient concentration of epinephrin to be detected by the rabbit intestine and uterus segments even where the eye reactions were negative. In all the experiments the rate of liberation was reduced by the nerve sections to a small fraction of the normal output.

<center>SURVIVAL EXPERIMENTS</center>

Seven of these are recorded here, all on cats. In all both eye reactions and segments tests were employed. One of the animals was tested 8 days after the nerve section (experiment 6); two after 16 and 15 days, respectively (experiments 7 and 8); one after 3 weeks (experiment 9); and three after 15 weeks (experiments 10, 11 and 12). In all the animals one superior cervical ganglion was removed about a week before the tests.

Experiment 6. Condensed protocol. Cat. Weight, 2.0 kgm. Right adrenal gland excised, nerve connections to left semilunar ganglion severed, and left superior cervical ganglion excised, 8 days before the experiment. Condition good.

9.30 a.m.   3 grams urethane.
10.30 a.m.   Tracheal cannula inserted, cava pocket made with cannula in lower end.
11.10 a.m.   Sample of jugular blood obtained.
11.25 a.m.   Pocket experiment, 1 minute, 30 seconds, no pupil or nictitating reaction.

11.28 a.m.   Pocket experiment, 2 minutes, no pupil or nictitating reaction.

11.32 a.m.   Pocket experiment, 3 minutes, questionable eye reactions.

11.37 a.m.   Pocket experiment, 4 minutes, pupil and nictitating reactions very faint, if any.

11.45 a.m.   Collected adrenal blood specimens as follows: First specimen, 1.7 grams in 3 minutes (0.57 gram per minute); second specimen, 3.4 grams in 7 minutes, 45 seconds (0.44 gram per minute); third specimen, 4.9 grams in 17 minutes (0.29 gram per minute); fourth specimen, 1.9 grams in 11 minutes, 30 seconds (0.16 gram per minute). Blood obtained from abdominal aorta with cava pocket still clipped off. Right adrenal weighed 0.165 gram, and contained 0.22 mgm. epinephrin. Left adrenal weighed 0.178 gram, and contained 0.27 mgm. epinephrin.
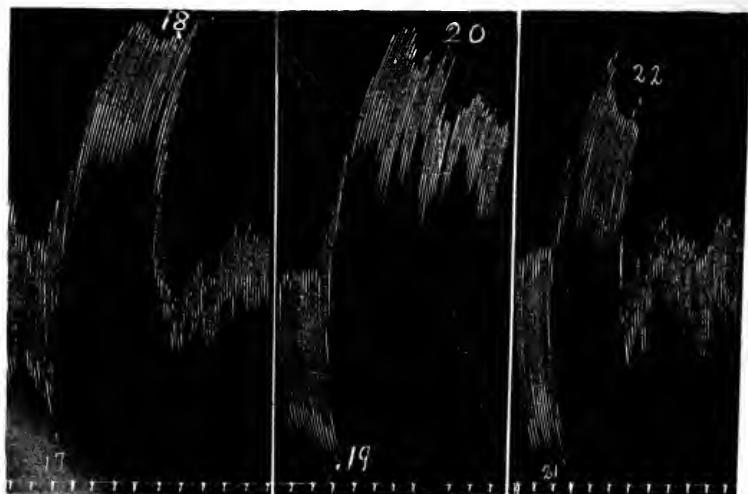
FIG. 8. INTESTINE TRACINGS. BLOODS FROM CAT WITH RIGHT ADRENAL EXCISED AND NERVES OF LEFT SEVERED

Anesthetized with urethane. At *17* Ringer was replaced by indifferent (arterial) blood, and this at *18* by the first adrenal specimen. At *19* Ringer was replaced by arterial blood and this at *20* by the second adrenal specimen. At *21* Ringer was replaced by arterial blood, and this at *22* by the third adrenal specimen. All bloods were diluted with an equal volume of Ringer before being applied to the segment. (Reduced to three-fifths.)

The eye reactions were practically negative. The segment tests proved that the concentration of epinephrin in the adrenal blood was very small even in the samples with the slowest blood flow. A few of the numerous tracings taken are reproduced in figures 8 and 9. Comparison of observations 20 and 28 indicates that the concentration in the second adrenal sample was not more
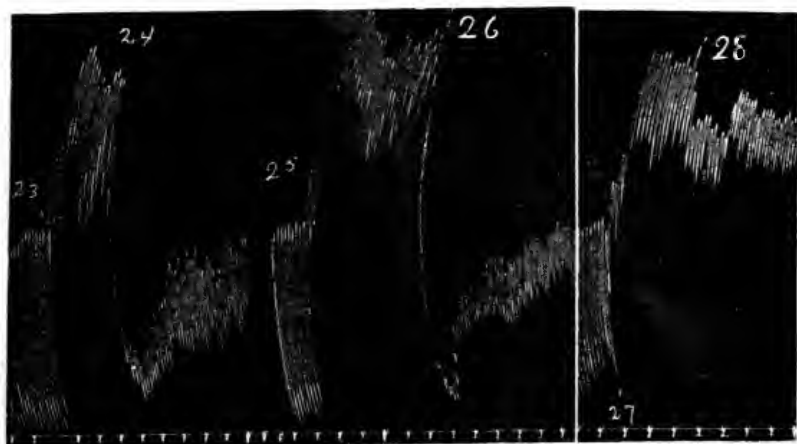
FIG. 9. INTESTINE TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURE 8

At 23 Ringer was replaced by arterial blood, and this at 24 by arterial blood containing 1 : 20,000,000 adrenalin. At 25 Ringer was replaced by arterial blood and this at 26 by arterial blood containing 1 : 50,000,000 adrenalin. At 27 Ringer was replaced by arterial blood, and this at 28 by arterial blood containing 1 : 125,-000,000 adrenalin. The indifferent (arterial) bloods were diluted with an equal volume of Ringer and after addition of the adrenalin to the arterial blood to make up the concentrations named, the adrenalin blood was also diluted with an equal volume of Ringer before application to the segments. (Reduced to three-fifths.)

than 1 : 125,000,000. It was very much less than 1 : 50,000,000 (observation 26). The intestine segment was quite sensitive to adrenalin. Indifferent blood made up with adrenalin to 1 : 50,-000,000, the mixture being then diluted with its own volume of Ringer before application to the segment, produced an effect probably not far from maximal as regards the initial diminution of tone. The greater effect of a 1 : 20,000,000 concentration

similarly diluted with Ringer (observation 24) is displayed simply in the greater persistency of the diminished tone. The third sample (observation 22) had a concentration of less than 1 : 50,000,000, probably no more than 1 : 75,000,000. The rates of flow of the second and third samples were in the ratio of 3 : 2, or approximately in the inverse ratio of the concentrations. It follows that the rate of liberation of epinephrin was proceeding uniformly during the collection of these samples. The greater concentration in the first adrenal sample may be assumed to be due to a small amount of epinephrin, the liberation of which was associated with the necessary manipulation in closing off the pocket. This would tell especially when the genuine output of epinephrin was very small, as in this experiment.

The uterus tests confirmed the conclusion that the inhibition of the intestine was due altogether to epinephrin.

Taking the rate of flow of the second sample as 0.43 cc. per minute, and the concentration of epinephrin in it as 1 : 125,000,000, we get 0.0000035 mgm. per minute as the output of epinephrin from the one adrenal, i.e., 0.000007 mgm. for the two adrenals (0.000003 mgm. per kilo of animal per minute). This is at most no more than one-hundredth of the normal output as determined by rabbit segments in adrenal blood (table 2), and not more than one-two-hundredth of the normal output as determined by the eye reactions (table 1).

Experiment 7. Condensed protocol. Cat. Weight, 2.75 kgm. The right adrenal gland was excised and the nerve connections to the left semilunar ganglion severed 2 weeks before the experiment. The left superior cervical ganglion was excised 9 days before the experiment. Condition excellent.

11.00 a.m.   4 grams urethane.
12.30 p.m.   Tracheal cannula inserted. Jugular blood obtained. Cava pocket made.
1.22 p.m.   Pocket experiment, 2 minutes. No pupil or nictitating reactions.
1.26 p.m.   Pocket experiment, 3 minutes, no pupil or nictitating reactions.

1.30 p.m. Collected the following adrenal blood specimens: First
specimen, 2.7 grams in 1 minute, 30 seconds (1.8 grams
per minute); second specimen, 5.7 grams in 3 minutes,
30 seconds (1.6 grams per minute); third specimen
7.4 grams in 7 minutes (1 gram per minute). Blood
obtained from abdominal aorta. Specific gravity of
this blood, 1028. The blood pressure during collection
of the adrenal specimens was good. Right adrenal
weighed, 0.110 gram, and contained 0.08+ mgm.
epinephrin. Left adrenal weighed, 0.214 gram, and
contained 0.18 mgm. epinephrin.

FIG. 10. INTESTINE TRACINGS. BLOODS FROM CAT WITH RIGHT ADRENAL
EXCISED AND NERVE CONNECTIONS OF LEFT CUT

Anesthetized with urethane. At *5* and *15* Ringer was replaced by indifferent
(arterial) blood, and this at *6* and *16* by the second and third adrenal specimens,
respectively. The bloods were all diluted with an equal volume of Ringer before
application to the segments. (Reduced to one-half.)

The eye reactions in experiment 7 were negative. Some speci-
mens of tracings from the segments tests are reproduced in
figures 10 and 11. From these and other observations on the
intestine segments, it was determined that the second adrenal
blood specimen (observation 6) was weaker than 1:30,000,000
adrenalin (observation 12), much weaker than 1:15,000,000

(observation 10). The third adrenal specimen (observation 16) had a greater concentration than the second, corresponding to the lessened blood flow, but it was still not as strong as 1 : 30,000-000. The amount of epinephrin liberated per minute was therefore not as much as 0.00003 mgm. for the one adrenal, or 0.00006 mgm. for both glands (0.00002 mgm. per kilo of animal per
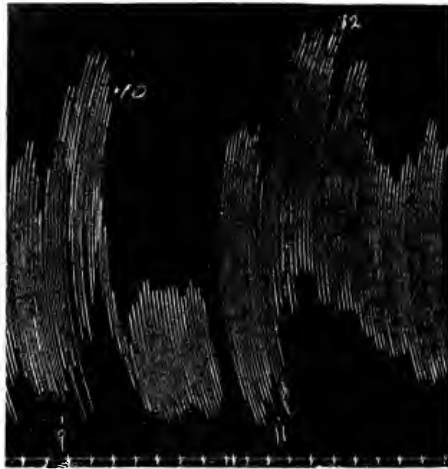
FIG. 11. INTESTINE TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURE 10

At *9* and *11* Ringer was replaced by arterial blood, and this at *10* and *12* by arterial blood to which had been added adrenalin to make up 1 : 15,000,000 and 1 : 30,000,000, respectively. All the bloods were diluted with an equal volume of Ringer before application to the segment. The concentrations of adrenalin given are the concentrations in the blood before this dilution. (Reduced to one-half.)

minute), i.e., not one-tenth of the normal discharge, as determined in drawn adrenal blood on rabbit segments, and not more than one-twentieth to one-thirtieth of the amount as determined by eye reactions.

The next two experiments of the survival series (experiments 8 and 9) yielded absolutely decisive results.

Experiment 8. Condensed protocol. Cat. Weight, 2.155 kgm. The right adrenal was excised and the nerve connections to the left

semilunar ganglion severed two weeks before the experiment. The left superior cervical ganglion was excised one week before the experiment. Condition good.

11.00 a.m.   4 grams urethane.

12.30 p.m.   Tracheal cannula inserted.   Cava pocket made.

1.05 p.m.   Pocket experiment, 45 seconds.   No eye reactions.

1.07 p.m.   Pocket experiment, 1 minute, 50 seconds. No eye reactions.

1.10 p.m.   Pocket experiment, 3 minutes 30 seconds. No eye reactions.

1.20 p.m.   Injected into jugular 0.5 cc. of 1 : 20,000,000 adrenalin. Very good pupil and nictitating reactions.

1.21 p.m.   Injected into jugular, 1.0 cc. of 1 : 20,000,000 adrenalin. Excellent pupil and nictitating reactions.

1.25 a.m.   Injected into jugular less than 0.5 cc.[3] of 1 : 20,000,000 adrenalin.   Good pupil and nictitating reactions.

1.30 p.m.   Inserted cannula in lower end of cava pocket, and collected the following adrenal blood specimens: First specimen, 2.0 grams in 1 minute, 15 seconds (1.6 grams per minute); second specimen, 1.9 grams in 1 minute, 45 seconds (1.1 grams per minute); third specimen, 3.7 grams, in 5 minutes, '10 seconds (0.7 gram per minute); fourth specimen, 2.8 grams in 7 minutes (0.4 gram per minute).   Blood obtained from jugular vein and also from abdominal aorta, while the cava pocket was still shut off from the circulation.

2.00 p.m.   Pocket experiment, 2 minutes.   No eye reactions.

2.03 p.m.   Pocket experiment, 3 minutes, 30 seconds. No eye reactions.

2.08 p.m.   Pocket experiment, 5 minutes.   No eye reactions.

2.20 p.m.   Injected into jugular 0.5 cc., of 1 : 40,000,000 adrenalin. Slight pupil reaction in 20.4 seconds.

2.23 p.m.   Injected into jugular 0.5 cc., of 1 : 40,000,000 adrenalin. Slight pupil reaction in 30 seconds.   Capacity of cava pocket slackly filled, 0.64 gram.   The arterial blood contained 72 per cent of serum (by hemaetocrit). Right adrenal weighed, 0.150 gram, and contained 0.25 mgm. epinephrin (assayed when excised).   Left adrenal weighed, 0.230 gram, and contained 0.24 mgm. epinephrin (assayed after the experiment).

[3] It was intended to inject 0.5 cc., but it did not wash in completely with the Ringer's solution (about 1 cc.) which followed the injection.

The eye reactions in experiment 8 were negative, although they could be elicited by injection of 0.00001 mgm. of adrenalin into the jugular vein, and were strongly obtained with less than 0.000025 mgm. Even with collection in the pocket for 5 minutes, no eye reactions were got, i.e., not even 0.000002 mgm. epinephrin per minute was being liberated by the one adrenal (i.e. 0.000002 mgm. per kilogram of body weight for the two adrenals). This is not more than one-three-hundredth of the normal output, as estimated by eye reactions.

A few specimens of the tracings from the rabbit intestine and uterus segment tests are given in figures 12 to 15. They showed that if any epinephrin whatever was present in the adrenal vein blood, which was not certain, it could only have existed in a concentration already almost beyond the limit of detectability by the extremely sensitive intestine and uterus segments worked with. For example, if the slight dip at observation 21 (fig. 13) is due to epinephrin, and not merely to the change of liquid around the preparation, it indicates that the concentration of epinephrin in the third adrenal blood specimen could not have been as much as 1 : 100,000,000 (observation 19). That with this segment a slight dip in the curve could be caused by the mere change of the blood surrounding the segment without any epinephrin being present, is illustrated in observation 9 (fig. 12), where arterial blood from one cat replaced arterial blood from another. Observation 17 (fig. 12) on the third adrenal sample reveals no definite inhibition. In observation 11, where the first adrenal blood replaced indifferent (arterial) blood, there is only a slight dip and a delayed increase of tone, notwithstanding the possibility that a little epinephrin might have been liberated into this sample by manipulation. Even the serum of the adrenal blood, which, as has been previously shown (3), is richer in epinephrin than the blood (containing, indeed, practically the whole of it), caused no definite inhibition of the intestine segments (fig. 14, observation 26), while 1 : 60,000,000 adrenalin in indifferent blood produced good inhibition (observation 29), and the effect of 1 : 100,000,000 (observation 31) was distinct.

On uterus segments, it was shown that adrenalin in the con-

centration of 1:200,000,000 (fig. 15, observations 41 and 45) caused a much greater increase of tone than the undiluted serum of the fourth adrenal specimen, notwithstanding the fact that the adrenalin was made up in an indifferent blood diluted with

FIG. 12. INTESTINE TRACINGS. BLOODS FROM CAT WITH RIGHT ADRENAL EXCISED AND NERVES OF LEFT CUT

Anesthetized with urethane. At *8* Ringer was replaced by arterial blood, and this at *9* by the same arterial blood. At *10* Ringer was replaced by arterial blood, and this at *11* by the first adrenal blood specimen. At *16* Ringer was replaced by arterial blood and this at *17* by the third adrenal specimen.   All the bloods were diluted before application to the segment with two volumes Ringer; except at observations 16 and 17, where the bloods were diluted with one volume Ringer.   (Reduced to two-thirds.)

its own volume of Ringer, and that the ordinary serum effect on the uterus would therefore be less than the serum effect in observation 43.   The indifferent blood in this dilution (tracings not reproduced), and also the adrenal vein blood of the second speci-

men (observation 38) similarly diluted, caused a much smaller effect on the uterus segment than the undiluted serum of the fourth adrenal specimen, and also a smaller effect than the indifferent blood (diluted with one volume Ringer) to which adrenalin in the concentration of 1: 300,000,000 (observation 44) had been added. It is probable .that even 1: 400,000,000 (observation 42) could be detected by this segment.

There is no doubt, then, that the serum of the fourth adrenal specimen contained . much less than 1: 200,000,000, and even less than 1: 300,000,000. The proportion of serum in the blood
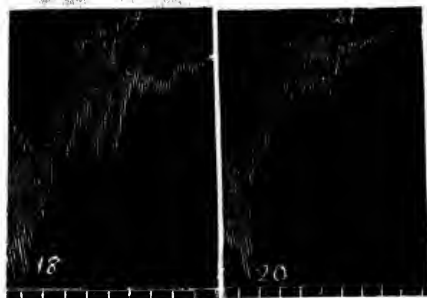
FIG. 13. INTESTINE TRACINGS. BLOODS FROM THE SAME CAT USED FOR FIGURE 12, BUT WITH SMALLER MAGNIFICATION

At *18* Ringer was replaced by arterial blood, and this at *19* by arterial blood to which had been added adrenalin to make up 1: 100,000,000. At *20* Ringer was replaced by arterial blood, and this at *21* ·by the third adrenal specimen. (Reduced to one-half.)

was 72 per cent (cat's blood is usually very rich in serum) so that the blood could not have had a greater concentration at most than 1: 400,000,000 of epinephrin. The rate of blood flow during collection of the fourth adrenal specimen was 0.4 gram ·per minute. The output of epinephrin per minute, accordingly, could not have been more than 0.000001 mgm. per minute for the one adrenal, or 0.000002 mgm. for the two adrenals (0.000001 mgm. per kilo of body weight per minute). This is no more than one-two-hundred and fiftieth of the normal output, as estimated in drawn adrenal blood under our experimental conditions on rabbit intestine and uterus segments, and no more
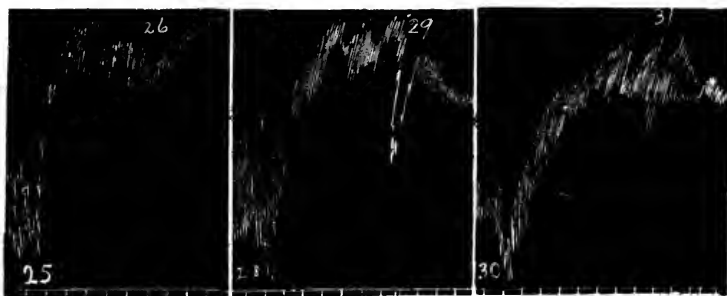
FIG. 14. INTESTINE TRACINGS. BLOOD FROM SAME CAT USED FOR FIGURES 12 AND 13

A fresh segment was taken. At *25* Ringer was replaced by serum of arterial blood, and this at *26* by serum of the fourth adrenal specimen. At *28* and *30* Ringer was replaced by arterial blood, and this at *29* and *31* by arterial blood to which adrenalin had been added to make up 1: 60,000,000 and 1: 100,000,000, respectively. (Reduced to one-half.)
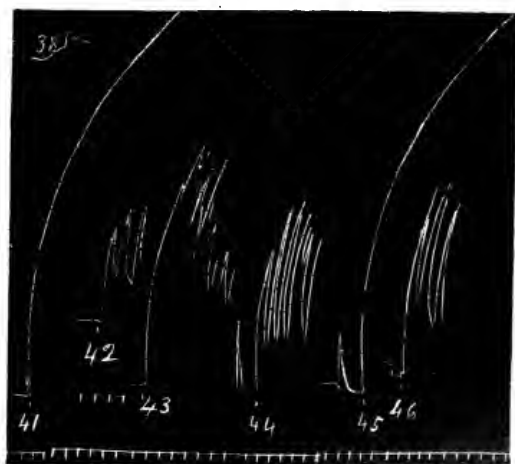
FIG. 15. UTERUS TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURES 12 to 14

At *38* Ringer was replaced by the second adrenal specimen diluted with an equal volume of Ringer; at *41* by arterial blood to which was added adrenalin 1:200,000,000 (writing point went above drum and stayed up a long time); at *42* by arterial blood, to which was added adrenalin 1: 400,000,000. The arterial bloods were diluted with an equal volume of Ringer before adding the adrenalin. At *43* Ringer was replaced by the serum of the fourth adrenal specimen undiluted; at *44* by arterial blood to which was added adrenalin (1: 300,000,000); at *44* by adrenalin, 1: 300,000,000; at *45* by adrenalin, 1: 200,000,000 (writing point went above drum and stayed up long); at *46* by adrenalin, 1: 300,000,000. The adrenalin was added in each case to arterial blood previously diluted with an equal volume of Ringer. (Reduced to two-fifths.)

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than one-five-hundredth of the normal output, as estimated by eye reactions.

Experiment 9. Condensed protocol. Cat. Weight 3.87 kgm. Three weeks before the experiment the right adrenal gland was excised and the left semilunar ganglion extirpated. The lumbar sympathetic chain was also cut and one lumbar ganglion below the diaphragm excised. The left superior cervical ganglion was excised 6 days before the experiment.

11.30 a.m.   Urethane 6 grams by stomach tube.

1.00 p.m.   Tracheal cannula inserted and jugular vein blood obtained.

1.20 p.m. to 1.45 p.m.   Cava pocket made, tying off renal, coeliac, mesenteric arteries and abdominal aorta.

1.50 p.m.   Pocket experiment. 1 minute, 35 seconds. No eye reactions.

1.52 p.m.   Pocket experiment. 3 minutes. No eye reactions.

The following specimens of adrenal blood were then collected:

NUMBER OF ADRENAL SPECIMENS	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE
	grams	minutes	seconds	grams
1	2.2	1	25	1.5
2	5.5	4		1.4
3	4.7	4		1.2
4	5.0	5		1.0
5	5.3	8		0.66
6	5.8	10	30	0.55

Obtained blood from carotid.

Right adrenal weighed 0.320 gram and contained 0.33 mgm. epinephrin. Left adrenal weighed 0.316 gram and contained 0.32 mgm. epinephrin.

The first adrenal specimen contained 55 per cent serum (hematocrite) an unusually small proportion of serum for a cat.

The eye reactions in experiment 9 were negative. Some of the tracings of the segment tests are reproduced in figures 16 to 19. None of the samples of adrenal blood gave any reactions indicating the presence of epinephrin, either with intestine or uterus, although the intestine could detect 1:300,000,000 adrenalin (fig. 17, observation 24). A concentration of 1:200,000,000

caused a marked effect on the intestine (observation 22). With the uterus employed it was proved that a concentration of 1: 300,000,000 was quite easily detectable (tracing not reproduced), and even 1: 500,000,000 (fig. 19, observation 49) could be detected.

In figure 16 it is shown that (in dilution 1: 2), even the sixth adrenal blood specimen, which normally would be relatively
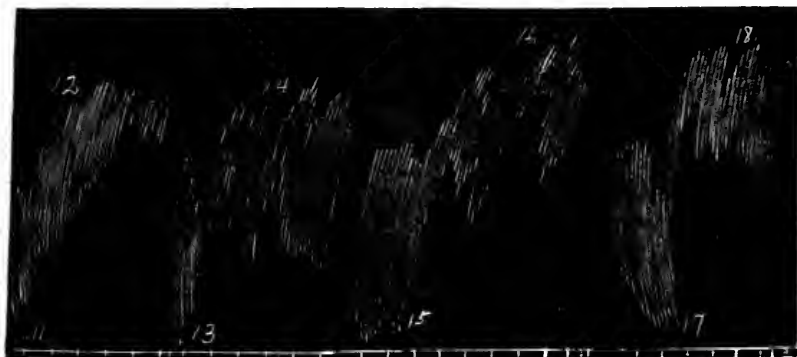
Fig. 16. Intestine Tracings. Bloods from a Cat with Right Adrenal Excised, Left Semilunar Ganglion Extirpated and Lumbar Sympathetic Chain Severed Three Weeks Before the Experiment

Anesthetized with urethane. At *11* Ringer was replaced by jugular blood and this at *12* by the sixth adrenal specimen. At *13* Ringer was replaced by jugular blood, and this at *14* by jugular blood to which was added adrenalin 1: 50,000,000. At *15* Ringer was replaced by jugular blood and this at *16* by the fourth adrenal specimen. At *17* Ringer was replaced by jugular blood and this at *18* by jugular blood to which was added adrenalin 1: 100,000,000. All the bloods, including the adrenalin bloods after being made up to the concentrations mentioned, were diluted with two volumes of Ringer before application to the segment. (Reduced to one-half.)

rich in epinephrin, owing to the small blood flow at the time of collection, caused no inhibition whatever (observation 12). The same was true of the fourth adrenal blood specimen (observation 16). The intestine segment gave a good reaction with adrenalin blood (1: 50,000,000) (observation 14), similarly diluted, and a distinct reaction with adrenalin blood (1: 100,000-000) (observation 18).

In figure 17, are displayed the results of some of the tests on intestine segments with undiluted blood. The sixth adrenal specimen gave no definite inhibition (observation 20). Indifferent blood to which adrenalin had been added to make up a concentration of 1 : 200,000,000, gave a good reaction (observation 22), and even a concentration of 1 : 300,000,000 caused distinct inhibition (observation 24). Even the serum (of the fifth adrenal specimen) caused no inhibition, but a considerable increase of tone of the intestine segment (fig. 18, observation 33). Adrena-
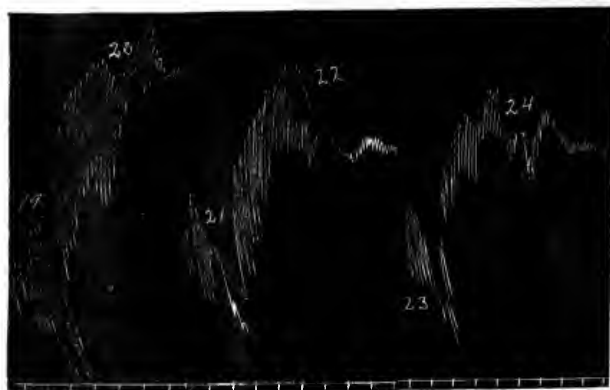
Fig. 17. Intestine Tracings. Blood from Same Cat Used for Figure 16

At *19* Ringer was replaced by indifferent (arterial) blood, and this at *20* by the sixth adrenal specimen. At *21* and *23* Ringer was replaced by arterial blood, and this at *22* and *24* by arterial blood to which had been added adrenalin to make up 1 : 200,000,000 and 1 : 300,000,000, respectively. (Reduced to one-half.)

lin in indifferent serum in concentration 1 : 300,000,000 produced a very different effect on the intestine (observation 35), and one which must be interpreted as an adrenalin action, that is to say, instead of a decided increase of tone, a preliminary slight inhibition followed by a recovery scarcely above the initial level.

The third adrenal blood specimen (diluted with 2 volumes Ringer) caused also a rise of tone, without inhibition (observations 28). All the other adrenal specimens were tested, but

none of them, not even the first, or so-called "manipulation" specimen, gave any positive effect.

The uterus tests confirmed the negative results obtained with the intestine. Specimens of the tracings are reproduced in figure 19. The third undiluted adrenal blood sample (observation 50), gave a somewhat smaller increase of tone than adrenalin
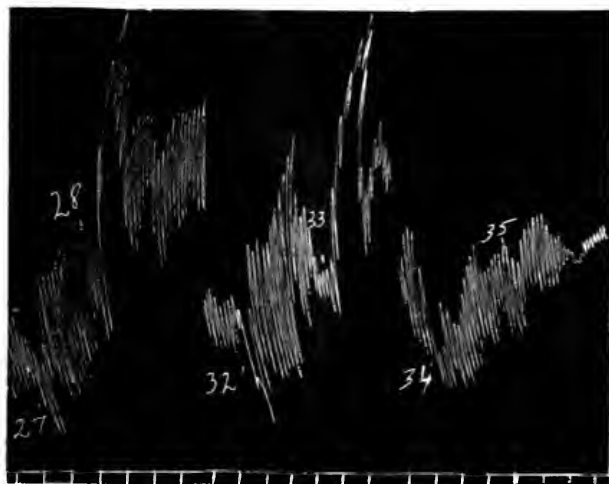
FIG. 18. INTESTINE TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURES 16 AND 17

At *27* Ringer was replaced by arterial blood and this at *28* by the third adrenal specimen, the bloods being diluted with two volumes Ringer before application to the segment. At *32* Ringer was replaced by serum of arterial blood and this at *33* by serum of the fifth adrenal specimen. At *34* Ringer was replaced by serum of arterial blood and this at *35* by serum of arterial blood to which adrenalin had been added to made up 1:300,000,000. All the sera were undiluted. (Reduced to two-thirds.)

added to the second adrenal specimen to make up a concentration of 1:500,000,000. The serum of the fifth adrenal blood sample (observation 51), produced a rise of tone of about the same size as this adrenalin blood, probably a little less. No difference between the indifferent bloods and the sixth adrenal blood specimen was brought out by diluting them to the same degree (observations 38 to 40).

There is evidence, then, from the intestine tests that the adrenal blood certainly did not contain 1: 300,000,000 epinephrin, and that even the serum in all probability did not contain 1: 300,000,-000. The uterus tests bring the possible concentration still lower, probably to 1: 500,000,000 for the serum. But even taking 1: 400,000,000 as the concentration which the serum could not have exceeded, we get for the blood (of the fifth adrenal specimen) a concentration of no more at most than 1: 700,000,000. The rate of flow during the collection of this specimen was 0.65
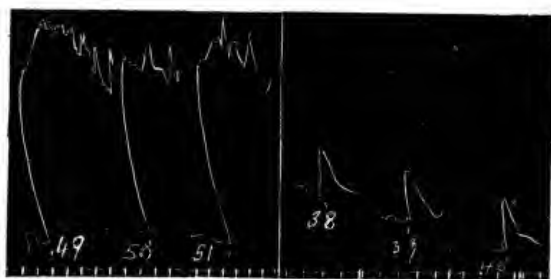
FIG. 19. UTERUS TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURES 16 TO 18

At *38* Ringer was replaced by arterial blood; at *39* by jugular blood; at *40* by the sixth adrenal blood specimen. The bloods were diluted with two volumes Ringer. At *49* Ringer was replaced by blood of the second adrenal specimen to which had been added adrenalin to make up 1: 500,000,000. At *50* Ringer was replaced by the third adrenal specimen; at *51* by serum of the fifth adrenal specimen. In observations 49 to 51 there was no dilution before application to the segment. (Reduced to one-half.)

gram (say 0.6 cc.) per minute. The rate of liberation of epinephrin accordingly could not have been as much as 0.0000009 mgm. per minute for the one adrenal (or 0.0000004 mgm. per kilogram of body weight per minute for two adrenals), i.e., not one-five-hundredth of the normal output, as estimated on drawn adrenal blood by rabbit segments, or one-thousandth of the normal output, under similar experimental conditions, as estimated by the eye reactions.

Results such as those of experiments 8 and 9 indicate that no epinephrin is normally liberated from the adrenals, except through

nerves. It must be remembered that the experimental conditions under which the blood samples were obtained are such as are usually considered to be most favorable for increasing the epinephrin output (anaesthesia, trauma, etc.)

Another conclusion which seems to follow is that the epinephrin normally liberated from the adrenals is not indispensable for life and health. For these cats had not suffered in any respect from the lack of it, so far as could be made out. If it be said that quantities and concentrations of epinephrin smaller than can be detected by the test objects employed, may still, for all we know, play an important part in the organism, the possibility may be conceded. But we do not know any functions which can be demonstrably affected by epinephrin in concentrations of 1:70 thousand millions (the adrenal blood is diluted at least one hundred times in the right heart). And reactions which can be demonstrated are the only reactions which can be studied.

No account is here taken of the possibility that the sporadic chromaffin tissue may discharge a certain amount of epinephrin, since although it has been shown to contain that substance (4) nothing is known as to its liberation. Accessory adrenals were always looked for *post mortem*, but were not present in any of our cats. In any case, accessory adrenals consist of cortical, not of medullary tissue.

It is scarcely necessary to say that our results do not show that no important or indispensable substance continues to be given off by the adrenals or even by the adrenal medulla after section of their nerves. On the contrary, as the adrenals are indispensable to life it must be assumed that the most important of their functions can be carried on through the blood or lymph in the absence of the nerves which control the liberation of epinephrin. The objection that the rate of liberation was not determined in these cats before section of the nerves and that it might have been exceptionally small, has no weight. For we do not find very wide variations in the output under our experimental conditions (table 2), and we never encounter a cat with intact adrenal nerves whose adrenal vein blood does not con-

tain epinephrin in concentration far above the limit of detect-
ability with test objects of normal sensitiveness.

The concentration in dog's adrenal blood is usually consider-
ably less than in the cat, probably due largely, if not entirely, to
the greater rate of blood flow.   It is for this reason easier to miss
detecting epinephrin in dog's adrenal vein blood than in cat's,
especially if the blood flow has not slackened considerably during
collection of a series of samples and if rather insensitive segments
happen to be employed.   This is undoubtedly the reason why
in previous experiments made by one of us (5) a negative
result was sometimes obtained in blood from the dog's adrenal
veins, with the nerves intact.   Occasionally also in the previous
experiments on dogs there was some admixture of the adrenal
blood with blood from the transverse lumbar vein, which would
still further reduce the epinephrin content.   It is not very un-
common in the dog, to find one adrenal vein, especially the left,
opening into the renal vein, and if this is not recognized in tying
off the cava pocket, the adrenal vein may easily be occluded.
Although this would not affect the concentration of epinephrin
in the blood collected, it would diminish the apparent rate of
liberation by half.

In three experiments, the cats were allowed to live for such a
time (15 weeks), as to permit at least the possibility of some
regeneration of the fibers.   These animals differed in no respect
in their behavior and state of health from the cats which were
allowed to survive for shorter periods.

Experiment 10.   Condensed protocol.   Cat.   Weight 2.06 kgm. at
the first operation.   The left adrenal gland was excised and the nerves
coming to the right semilunar ganglion severed 15 weeks before the
experiment.   The left superior cervical ganglion was excised one week
before the experiment.   Body weight 2.63 kgm. at the time of the
experiment.   Condition good.

10.40 a.m.   Ether; cava pocket tied off, renal, coeliac, mesenteric
            arteries and abdominal aorta below the renals being
            ligated.

11.10 a.m.   Pocket experiment 52 seconds.   No eye reactions.

11.15 a.m.   Pocket experiment 1 minute, 32 seconds. No eye
              reactions. About 10 cc. blood was obtained from the
              jugular vein.
11.30 a.m.   Pocket experiment 1 minute, 30 seconds. No eye re-
              actions.
11.35 a.m.   Pocket experiment 1 minute, 50 seconds. No eye reac-
              tions. Tracheal cannula inserted, also cannula in
              lower end of pocket, in iliac vein.
11.52 a.m.   Adrenal blood collected from pocket. Flow very slow,
              about 5 cc. in 27 minutes. Obtained blood from
              right heart. Capacity of pocket, slackly filled, 0.39
              gram. Left adrenal weighed 0.346 gram and contained
              0.22 mgm. epinephrin. Right adrenal weighed 0.330
              grams and contained 0.22 mgm. epinephrin. The
              bloods were centrifuged and the serums tested.

The eye reactions for epinephrin in the adrenal blood were
negative. With the uterus segments (fig. 20), no evidence of
the presence of epinephrin even in the serum of the adrenal vein
blood was obtained, although the blood flow during the collec-
tion of the specimen was slow. The adrenal serum (diluted with
5 volumes Ringer), caused scarcely as great an increase of tone
as the jugular serum similarly diluted (observations 19 and 20).
With a smaller degree of dilution (1:3), adrenal serum was dis-
tinctly inferior to jugular in tone-increasing power. The rise
caused by the undiluted adrenal serum (observation 31), on a
different uterus segment, was much smaller than that due to
an adrenalin solution (1:30,000,000) in an indifferent serum
(diluted with 3 volumes Ringer) (observation 30). There was
evidence that the rise of tone caused by the adrenalin solution
was maximal for this segment. So that the uterus could un-
doubtedly have detected a far smaller concentration of adrenalin
than 1:30,000,000. The indifferent serum in which the adrenalin
solution was made up (diluted with 3 volumes Ringer), caused
a somewhat greater rise than the undiluted adrenal serum.

The great difference in tone-increasing power between the
adrenal and the indifferent sera in this experiment suggests that
the former contained some substance which caused inhibition

of uterus tone. It is known that such a substance is sometimes present in venous blood, during asphyxial conditions. For this reason it is certain that the inhibitory effects obtained on the intestine segment were not due, at least wholly, to epinephrin. It is not often that this circumstance complicates the estimation of the concentration of epinephrin by the segments (this experiment, indeed, is the only instance in the series recorded in this paper). But it can only be controlled by using both uterus and

Fig. 20. Uterus Tracings. Bloods from Cat with Left Adrenal Excised and Nerve Connections of Right Semilunar Ganglion Cut Fifteen Weeks before Experiment

Anesthetized with urethane. At *19* Ringer was replaced by serum of adrenal blood (a small sample slowly collected), at *20* by serum of jugular blood; both diluted with five volumes Ringer. At *29* (with another segment) Ringer was replaced by serum of a dog diluted with three volumes Ringer; at *30* by dog serum to which adrenalin had been added to make up 1 : 10,000,000, the adrenalin serum being then diluted with three volumes Ringer before application to the segment. At *31* Ringer was replaced by undiluted serum of the cat's adrenal blood. (Reduced to one-half.)

intestine segments for the tests. Even if the inhibition of the intestine (figure 21) caused by the adrenal serum (observation 6) were entirely due to epinephrin, the concentration in the serum must have been much less than 1 : 10,000,000 (observation 14), and in the blood much less than 1 : 17,000,000. If we take the concentration in the blood even at 1 : 20,000,000 this would correspond to an epinephrin output of no more than 0.00001 mgm. per minute for one adrenal (or 0.000007 mgm. per kilo-

gram of body weight per minute for the two adrenals).   This
is not more than one-thirtieth to one-fortieth of the normal
output as estimated on drawn adrenal blood on rabbit segments,
or one-hundredth of the normal as estimated by the eye reactions.

FIG. 21. INTESTINE TRACINGS.   BLOODS FROM SAME CAT USED FOR FIGURE 20

At *1* Ringer was replaced by serum of jugular blood and this at *2* by serum of
adrenal blood.   The sera in observations 1 and 2 were undiluted.   At *5* Ringer
was replaced by jugular serum and this at *6* by adrenal serum, both diluted with
an equal volume of Ringer.   At *9* Ringer was replaced by dog serum diluted with
an equal volume of Ringer and this at *10* by the same serum made up to 1 : 3,000,-
000 with adrenalin, and then diluted with an equal volume of Ringer before appli-
cation to the segment.   At *13* Ringer was replaced by jugular serum and this at
14 by jugular serum to which adrenalin had been added to make up 1 : 10,000,000.
Both jugular serum and adrenalin serum were diluted with an equal volume of
Ringer before application to the segment.   (Reduced to one-half.)

Experiment 11. Condensed protocol. Cat. Weight 3.06 kgm. at
the time of the operation, 4.14 kgm. at the time of the experiment.
The right adrenal gland was excised and the nerves coming to the left
semilunar ganglion cut 15 weeks before the experiment. The left
superior cervical ganglion was excised 8 days before the experiment.
Condition excellent.

11.30 a.m.   4 grams urethane.

1.00 p.m.   Inserted tracheal and jugular cannulae.   Obtained sample of jugular blood.

1.20 p.m.   Made cava pocket, with cannula in a renal vein.

2.05 p.m.   Pocket experiment 2 minutes, 10 seconds.   Dubious reaction of left pupil and nictitating.

2.15 p.m.   Pocket experiment 4 minutes.   Slight dilatation of pupil in 16 to 18 seconds; retraction of nictitating about 10 seconds later.

2.30 p.m.   Collected blood from pocket through cannula.   Poor flow.   About 2 cc. of adrenal blood collected in 20 minutes.

2.55 p.m.   Isolated left sympathetic in thorax on a loose ligature.

3.00 p.m.   Pocket experiment, 2 minutes, 35 seconds.   Small dilatation of pupil in 26 seconds, nictitating following 10 to 12 seconds later.

3.06 p.m.   Pocket experiment 3 minutes, 30 seconds.   Small pupil dilatation in 16 to 18 seconds, and nictitating about 12 seconds later.

3.13 p.m.   Tied and cut left sympathetic in thorax.

3.15 p.m.   Pocket experiment 4 minutes.   Slight retraction of nictitating in 32 seconds.   Pupil reaction doubtful.

3.21 p.m.   Pocket experiment, 5 minutes.   Very slight eye reactions.

3.29 p.m.   Peripheral end of left sympathetic stimulated for 25 seconds with pocket open.   Slight instantaneous dilatation of pupil.   No other pupil reaction.

3.35 p.m.   Stimulated left sympathetic 54 seconds.   No eye reactions.

3.37 p.m.   Pocket experiment 4 minutes.   No eye reactions.

3.51 p.m.   Pocket experiment with stimulation of left sympathetic 4 minutes.   Very slight but definite pupil dilatation in 44 seconds after release of the pocket, very slight retraction of nictitating following about 9 seconds later. Inserted cannula in lower end of pocket and obtained a small sample of adrenal blood.   Right adrenal weighed 0.186 gram and contained 0.23 mgm. epinephrin. Left adrenal weighed 0.190 gram and contained 0.12 mgm. epinephrin.

In experiment 11, slight but positive eye reactions were obtained, indicating a much reduced, but still detectable liberation

of epinephrin.   The first adrenal blood sample was found, by the the segment tests, to contain approximately 1:5,000,000 epinephrin.   This concentration would be rather low for a cat, even with a normal blood flow, and is very low for the small flow when the sample was collected.   The output per minute could not have been more than 0.00002 mgm. for the one adrenal (or 0.00001 mgm. per kilogram of body weight per minute for the two adrenals).   This is not more than one-twenty-fifth of the normal output as estimated on drawn adrenal blood by rabbit segments, and no more than one-fiftieth or one-sixtieth of the normal as estimated by the eye reactions.   It is worthy of note that the section of the left sympathetic in the thorax diminished the already slight eye reactions and stimulation of the nerve increased them slightly, but definitely.   In this cat, then, some of the secretory fibers in the sympathetic were capable at this time of conduction.   It is, of course, impossible to say whether these were regenerated fibers or fibers which had escaped section when the nerves coming to the semilunar ganglion were cut.

Experiment 12.   Condensed protocol.   Cat.   Weight at first operation 2.09 kgm., at the time of experiment 3.635 kgm.   The right adrenal was excised and the nerves coming to the left semilunar ganglion cut 15 weeks before the experiment.   The left superior cervical ganglion was excised 6 days before the experiment.   Condition excellent.

11.00 a.m.   Urethane 4 grams.
 1.30 p.m.   Tracheal cannula inserted.   Pocket made.
 2.10 p.m.   Pocket experiment, 1 minute, 30 seconds occlusion.   Good pupil reaction in 12 seconds after release of pocket, nictitating 4 seconds later.
 2.27 p.m.   Pocket experiment, 2 minutes.   Pupil reaction in 14 seconds, nictitating 10 seconds later.
 2.31 p.m.   Pocket experiment, 2 minutes.   Good pupil reaction in 14 seconds, nictitating 10 seconds later.

Collected adrenal blood through cannula in lower end of pocket. Flow very slow.   Poor circulation.   About 3 cc. collected in 26 minutes.   Isolated left sympathetic in thorax on loose ligature.

 3.25 p.m.   Pocket experiment, 2 minutes.   Slight pupil and nictitating reactions.

3.32 p.m.   Pocket experiment, 2 minutes, 30 seconds. Pupil re-
            action in 28 seconds, nictitating 6 seconds later. Tied
            and cut left sympathetic in the thorax.

3.40 p.m   Pocket experiment, 3 minutes. Slight pupil reaction in
            24.5 seconds. No nictitating. Cut right sympathetic
            in thorax.

3.50 p.m.   Pocket experiment, 3 minutes.· Slight pupil reaction in
            26 seconds. No nictitating.

3.55 p.m.   Pocket experiment, with stimulation of left sympathetic
            3 minutes. About the same reaction as at 3.50 (in
            26 seconds).

4.00 p.m.   Pocket experiment, 3 minutes. Slight pupil reaction in
            30 seconds. No nictitating.

4.05 p.m.   Pocket experiment, with stimulation of left sympathetic,
            3 minutes. Smaller pupil reaction than at 4.00, in
            36 seconds. Blood obtained from abdominal aorta,
            with pocket still clipped off. Right adrenal weighed
            0.150 gram and contained 0.16 mgm. epinephrin. Left
            adrenal weighed 0.164 gram and contained 0.09 mgm.
            epinephrin.

The eye reactions were positive in experiment 12. They were
not diminished by section of the sympathetics in the thorax,
nor were they increased by stimulation of the left sympathetic.
In this cat, there was no evidence that any of the secretory
fibers in the sympathetic were at this stage capable of conduc-
tion. The intestine segment tests showed that the adrenal
vein blood contained much less than $1:3,000,000$ epinephrin
(fig. 22), and not far from $1:6,000,000$ (fig. 23). The uterus
tests confirmed this (fig. 24). For instance, the adrenal blood
diluted with 5 volumes Ringer (observation 19), gave a some-
what smaller increase of tone than $1:6,000,000$ adrenalin made
up in indifferent blood and similarly diluted. The flow during
collection of the adrenal specimen was slow, doubtless consider-
ably slower than during the eye tests. The output of ·epi-
nephrin per minute was 0.00002 mgm. for the one adrenal
(or 0.00001 mgm. per kilogram of body weight per minute for
the two adrenals). This is not more than one-twentieth of
the normal output, as estimated on shed adrenal blood by
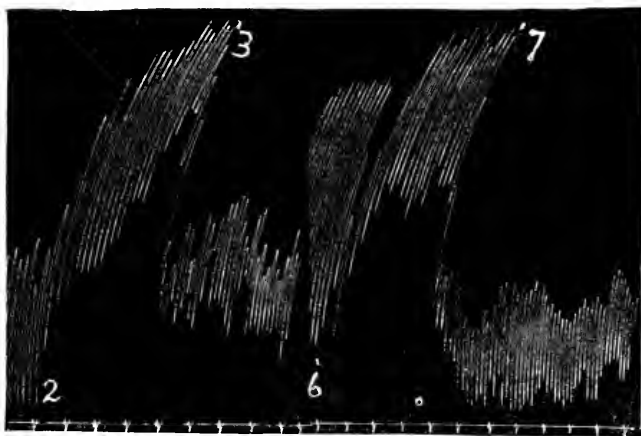
FIG. 22. INTESTINE TRACINGS. BLOODS FROM CAT WITH RIGHT ADRENAL
EXCISED AND NERVE CONNECTIONS OF LEFT SEMILUNAR GANGLION
CUT FIFTEEN WEEKS BEFORE EXPERIMENT

Anesthetized with urethane.   At *2* Ringer was replaced by arterial blood, and
this at *3* by adrenal blood (a small sample very slowly collected).   At *6* Ringer
was replaced by arterial blood and this at *7* by arterial blood containing,
1 : 3,000,000 adrenalin.   (Reduced to two-thirds.)
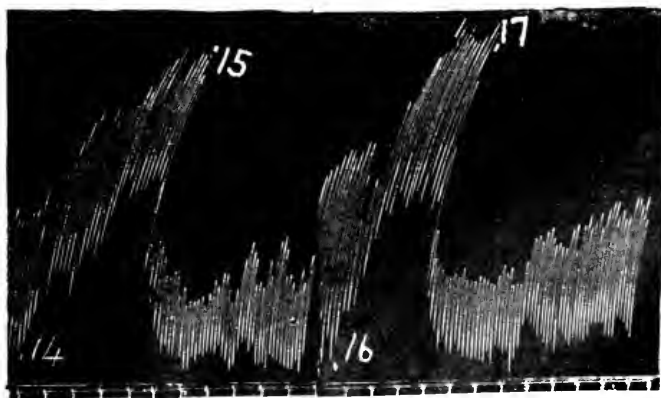
FIG. 23. INTESTINE TRACINGS.   BLOODS FROM SAME CAT USED FOR FIGURE 22

At *14* Ringer was replaced by arterial blood and this at *15* by adrenal blood,
both diluted with an equal volume of Ringer.   At *16* Ringer was replaced by ar-
terial blood diluted with one volume Ringer, and this at *17* by arterial blood to
which adrenalin had been added to make up 1 : 6,000,000, the adrenalin blood being
diluted with one volume Ringer before application to the segment.   (Reduced
to two-thirds.)

FIG. 24. UTERUS TRACINGS. BLOODS FROM SAME CAT USED FOR FIGURES 22
AND 23

At *19* Ringer was replaced by adrenal blood diluted with five volumes Ringer;
at *20* by arterial blood to which had been added adrenalin to make up 1 : 6,000,000,
the adrenalin blood being then diluted with five volumes Ringer before appli-
cation to the segment. (Reduced to one-half.)

rabbit intestine segment tests, and not more than one-fortieth
of the output, as estimated by eye reactions.

SUMMARY

1. We showed in a previous paper, by the blood pressure and
eye reactions, that after section of the nerve supply of the adrenal
no demonstrable liberation of epinephrin was present in cats as
long as five weeks after the nerve section.

2. As it is easier to detect very small concentrations of epi-
nephrin by the rabbit intestine and uterus segments, we have made
a series of survival experiments in cats in which these tests were
used to supplement the eye reactions. In all the animals one
adrenal was excised and the nerves of the other cut.

In a cat tested two weeks after the operation, it was shown
that the adrenal blood serum could not have contained 1 : 300-
000,000, or the blood 1 : 400,000,000 of epinephrin; and that the
rate of liberation of epinephrin could not have been at most
0.000001 mgm. per minute for one adrenal. In another cat
three weeks after the operation the serum of the adrenal blood
was proved to contain less than 1 : 400,000,000 and the blood

less than 1: 700,000,000 epinephrin. The output of epinephrin per minute could not have been as much as 0.0000009 mgm. per minute, for one adrenal. The segments used for the tests in these experiments were extremely sensitive, and the limits of adrenalin concentrations which could be detected with certainty were carefully determined. The eye reactions were negative. In these two cats the rate of liberation of epinephrin, if any liberation whatever was going on, must have been several hundred times less than the rate in normal animals under the same experimental conditions.

It is scarcely necessary to point out that experiments yielding completely negative results indicating the absence of epinephrin with very sensitive test objects are much more important for the questions studied than experiments in which small amounts of epinephrin can still be detected. For it is impossible to be certain that when a little epinephrin is found some of the fibers concerned in the liberation may not have escaped section.

3. Since these animals had completely recovered from the operation and behaved in every way like normal animals, it must be concluded that the liberation of epinephrin from the adrenals is not indispensable for life or health, unless indeed the necessary quantity is, even in the adrenal vein blood, below the limits of detection by the methods used. The epinephrin in the adrenal blood is diluted enormously (probably at least one hundred times) in the right heart; so that in these cats the concentration in the arterial blood could not, at most have reached 1: 40,000,000,000 and 1: 70,000,000,000, respectively.

If the liberation of epinephrin is totally abolished by division, in the dorsal cord, of the path concerned in it, as our experiments[4] on the Relation of the Spinal Cord to the Spontaneous Liberation of Epinephrin indicate, this corroborates the conclusion that epinephrin is not indispensable, since numerous animals and men have long survived such lesions.

4. The experiments indicate that the entire liberation of epinephrin from the adrenals is controlled by nerves.

[4] Proc. Soc. Exp. Biol. and Med., April 18, 1917; Jour. of Exper. Med., xxvi, 1917.

5. In some of the other cats the residual output of epinephrin was so small that it was doubtful whether it was being liberated at all in detectable amount.   In all, the rate of liberation, even where a definite output could still be detected, was reduced to a small fraction of the normal.

6. In a number of acute experiments on cats and dogs, the reduction in the output of epinephrin after section of the various possible nerve paths to the adrenals was studied.   In all, epinephrin was still found in detectable amount in the blood coming from the adrenals, although the rate of liberation was reduced to a small fraction of the initial amount.

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THE INFLUENCE OF ASPHYXIA UPON THE RATE OF LIBERATION OF EPINEPHRIN FROM THE ADRENALS[1]

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The hyperglycaemia and glycosuria associated with asphyxia have been explained by some writers as due to stimulation of the adrenals to increased liberation of epinephrin. This, however, is a hypothesis unsupported by any conclusive evidence showing that in asphyxia the rate at which epinephrin is discharged is, as a matter of fact, increased. Since it has been proved that the liberation of epinephrin from the adrenals is under the control of nerves, it would seem probable that asphyxia, which causes excitation of so many nervous centers, might excite the central mechanism on which the epinephrin secretion depends. We endeavored to put the question to the test by collecting adrenal blood in a vena cava pocket, and then by releasing the pocket permitting it to elicit the reactions appropriate to epinephrin on the blood pressure. In cases in which the pupil was not too greatly dilated by the asphyxia the (denervated) eye reactions, after excision of the superior cervical ganglion according to the procedure of Meltzer, were also employed. The results were negative (1). No clear difference could be made out in the magnitude of the reactions, in cats, when adrenal blood was collected in the pocket for equal times with and without asphyxia. As the epinephrin is, of course, greatly diluted before it reaches

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[1] A preliminary account of the work was given at the joint meeting of the Federation of American Societies for Experimental Biology, December 28, 1916. A note was published in the Proceedings of the Society for Experimental Biology and Medicine, January 17, 1917.

the sensitive structures concerned in the blood pressure and eye reactions, we have repeated the observations with unmixed adrenal blood withdrawn from the cava pocket by a cannula and tested upon rabbit intestine and uterus segments according to the method introduced by one of us (2). Specimens of adrenal blood were collected for accurately measured periods of time, with free and with obstructed respiration. Since when the adrenal blood flow is diminishing in successive samples, the concentration of epinephrin goes on increasing, the asphyxial and non-asphyxial periods did not follow each other in any definite order, so that an increase of concentration due merely to the diminution in the blood flow might not simulate an increase due to asphyxia. Special weight was also given to observations in which the successive samples, with and without asphyxia, were collected with unchanged rate of blood flow. Experiment 1 is an example of such experiments.

Experiment 1. Condensed protocol. Cat (male). 3.41 kgm., weight. Urethane. A sample of indifferent blood was obtained from the jugular vein. Then a cava pocket ("short" pocket) was made, the renal, coeliac, mesenteric arteries and abdominal aorta below the renals being tied. The following adrenal blood samples were then collected.

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	grams	*minutes*	*seconds*	*grams*	
1	2.0		50	2.2	No asphyxia
2	2.9	1	15	2.3	Asphyxia
3	3.1	1	20	2.4	Asphyxia
4	3.3	1	45	1.9	Without asphyxia
5	4.0	2		2.0	Without asphyxia
6	3.2	2		1.6	Asphyxia
7	2.0	1		2.0	Without asphyxia
8	6.5	4	25	1.5	Without asphyxia

Blood was now obtained from the abdominal aorta. Combined weight of adrenals, 0.550 gram.

In figure 1, it will be seen that the sixth adrenal specimen (collected during asphyxia) and the fifth specimen (collected

without asphyxia) caused almost the same amount of inhibition of the intestine. The same thing is seen in figure 2, where the two specimens were compared in a different dilution. If the sixth specimen has a slightly greater effect this is certainly no more than would be associated with the somewhat smaller rate of blood flow when the sixth sample was being collected. If asphyxia is capable of stimulating the adrenal to increased
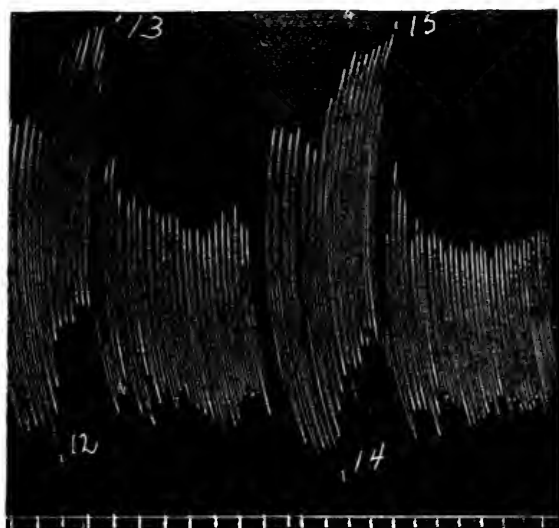
FIG. 1. INTESTINE TRACINGS.   BLOOD FROM CAT ANESTHETIZED WITH URETHANE

At *12*, Ringer was replaced by jugular blood, and this at *13* by the fifth adrenal blood specimen, collected without asphyxia. At *14*, Ringer was replaced by jugular blood, and this at *15* by the sixth adrenal blood specimen, collected during asphyxia. The bloods were diluted with six volumes of Ringer. As in all the tracings, the time is marked in half minutes.   (Reduced to two-thirds.)

secretion of epinephrin, a marked preponderance of epinephrin concentration ought to have been seen in the sixth specimen. In figure 3, the first adrenal specimen (collected without asphyxia) is seen to produce at least as great an effect as the third specimen (collected during asphyxia). The blood flows being practically equal during the collection of these two specimens, it can be assumed that the concentrations of epinephrin in them would

have been equal had they been both collected during free respiration. The obvious conclusion is that asphyxia produced either no effect whatever on the rate of output of epinephrin; or so small an effect, that this was below the threshold of detectability with the intestine segments employed. This conclusion was confirmed on rabbit uterus segments (fig. 4). The fifth adrenal specimen caused quite as great an increase of tone as the third,
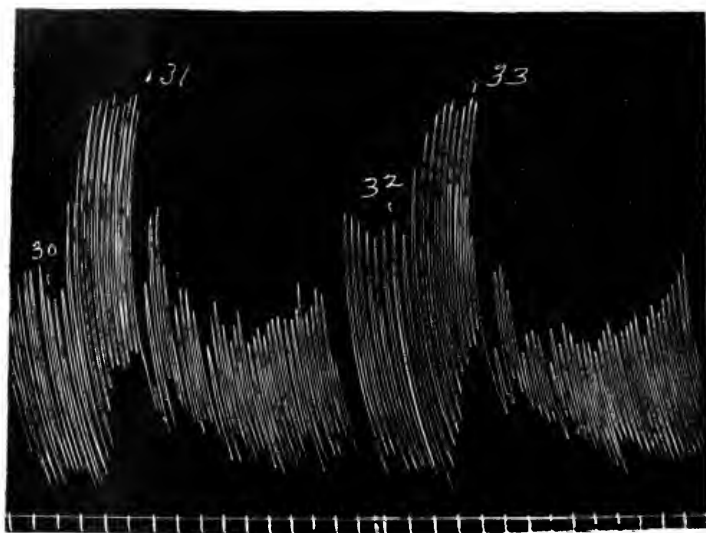
FIG. 2. INTESTINE TRACINGS. BLOOD FROM SAME CAT USED FOR FIGURE 1

At *30* Ringer was replaced by jugular blood, and this at *31* by the sixth adrenal specimen (asphyxia). At *32* Ringer was replaced by jugular blood, and this at *33* by the fifth adrenal specimen (without asphyxia). Bloods diluted with eight volumes Ringer. (Reduced to two-thirds.)

and the sixth specimen showed no definite preponderance over the fifth. An increase in adrenalin concentration from 1:3,000,-000 (fig. 4, observation 40) to 1:2,000,000 (observation 41) could have been easily detected by the uterus segment, and no doubt a much smaller difference.

Other experiments of this type on cats yielded a similar result. No clear evidence was obtained in any of them from a comparison
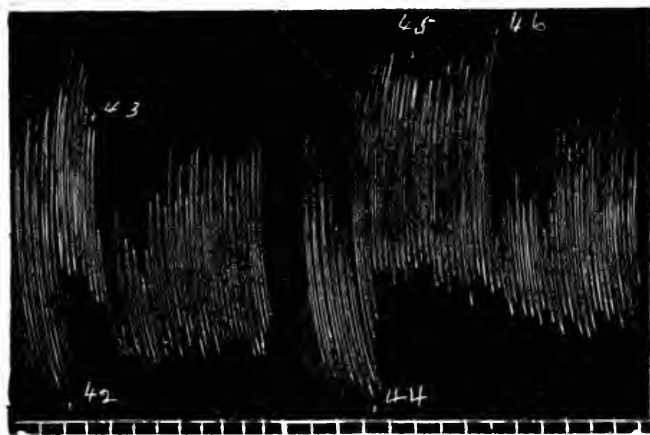
FIG. 3. INTESTINE TRACINGS.   BLOOD FROM SAME CAT USED FOR FIGURES 1 AND 2

At *42* Ringer was replaced by jugular blood, and this at *43* by the first adrenal blood specimen (without asphyxia).   At *44* Ringer was replaced by jugular blood. At *45* some more jugular blood was added, which was replaced at *46* by the third adrenal specimen (asphyxia).   Bloods diluted with eight volumes Ringer. (Reduced to two-thirds.)
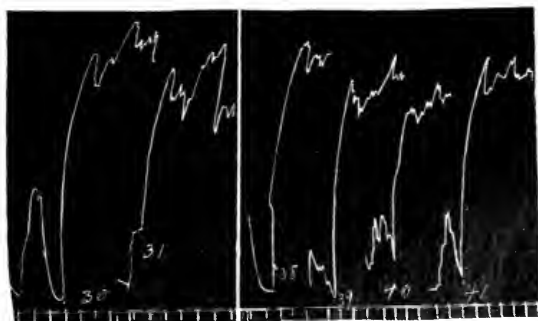
FIG. 4. UTERUS TRACINGS WITH BLOOD FROM CAT USED FOR FIGURES 1 TO 3

At *30* Ringer was replaced by the fifth adrenal blood specimen (without asphyxia); at *31* by the sixth adrenal specimen (asphyxia).   Both bloods were diluted with twenty volumes Ringer.   At *38* Ringer was replaced by the fifth adrenal specimen; at *39* by the third adrenal specimen (asphyxia).   Both bloods diluted with nine volumes Ringer.   At *40* adrenalin in carotid blood (1: 3,000,000) and at *41* adrenalin in carotid blood (1: 2,000,000) replaced Ringer.   Both adrenalin bloods, after being made up to the concentrations mentioned, were diluted with nine volumes Ringer before application to the segment.   The weight was increased between observations *31* and *38*.   (Reduced to one-half.)

of adrenal bloods collected during and without asphyxia that the rate of epinephrin output was sensibly increased by asphyxia. In one of these experiments on a cat anesthetized with urethane, adrenal blood collected during asphyxia was compared with adrenal blood collected during stimulation of sensory nerves (sciatic) (3), and both specimens with adrenal blood collected during free respiration and without sensory stimulation. No unequivocal difference in the rate of liberation of epinephrin in the different samples could be made out.

In the next experiment a dog was employed, to insure such a large flow of blood that the dead space in the cannula and cava pocket would be very quickly washed out between successive samples. In the cats, the "overlapping" of the asphyxial and non-asphyxial specimens was reduced to a minimum by beginning the asphyxia a little before the completion of collection of the preceding non-asphyxial specimen, and stopping it a little before the end of the collection of the asphyxial specimen. From the very large blood flows into the pocket in experiment 2, it is possible that some small vein going to the pocket was left untied, although no evidence of this was found at the end of the experiment. It is easier in the cat than in the dog to be certain that nothing has escaped ligation. However, in connection with the relatively large blood flow commonly observed in these experiments, both in cats and dogs, it must be noted that usually the blood pressure was quite high during a considerable part of the experiment, since so many arteries (renal, coeliac, mesenteric and abdominal aorta) were tied off. Whether a small leak existed or not in this dog makes no difference to the result of the experiment, since it would affect all the samples proportionally.

Experiment 2. Condensed protocol. Dog (female). Weight, 7.5 kgm. Ether. Cava pocket made (renal, coeliac, mesenteric and both iliac arteries tied). Cannulae inserted into each iliac vein. Indifferent blood obtained from jugular vein. The following specimens of adrenal blood were then collected from the cava pocket:

The first four specimens were collected through the right iliac cannula, and the last three through the left. While clipping off the right

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	cc.	minutes	seconds	cc.	
1	8.05		20	24.0	Asphyxia begun
2	37.1	2		18.5	Asphyxia
3	30.5	2		15.2	Without asphyxia
4	23.5	1	47	13.4	Without asphyxia
5	35.0	2	5	16.6	Asphyxia
6	23.0	2	20	10.0	Without asphyxia
7	14.3	2	15	6.35	Without asphyxia

and releasing the left the pocket filled up, and the amount of blood collected in the fifth specimen is greater than it should have been. Thus the greater flow for the fifth specimen given in the table is only apparent.   The epinephrin concentration would of course not be affected by this as it remained occluded at the upper end.   Combined weight of adrenals, 0.96 gram.
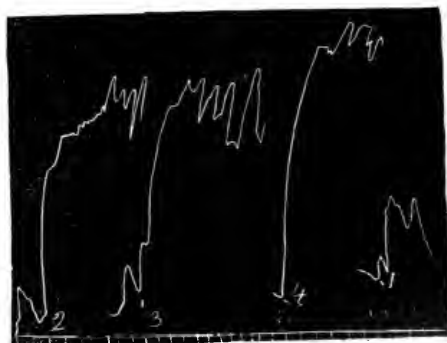
FIG. 5. UTERUS TRACINGS

At *1* Ringer was replaced by carotid blood from a dog; at *2* by the second adrenal blood specimen from the dog, collected during asphyxia; at *3* by the third adrenal blood specimen, collected without asphyxia; at *4* by the fourth adrenal specimen, collected without asphyxia.   All bloods diluted with four volumes Ringer.   (Reduced to one-half.)

In figure 5, some of the uterus tracings from experiment 2 are reproduced.   Observations 2 and 3 show that the third adrenal sample (collected without asphyxia) is at any rate as rich in epinephrin as the second (collected during asphyxia).   Intestine

segment tracings, not reproduced, demonstrated that the third specimen contained, indeed, somewhat more epinephrin than the second, which agrees with the slightly greater blood flow during collection of the second specimen. The progressive increase in concentration in successive samples was well shown on the uterus segments, for the adrenal specimens from the fifth to the seventh, despite the fact that the sixth and seventh samples were collected without asphyxia and might therefore have been expected to possess a smaller content of epinephrin than the fifth sample, had asphyxia been capable of increasing the output to an extent detectable by these methods.

It might be objected that under the experimental conditions (anesthesia, trauma, etc.) the output of epinephrin was so stimulated that it was already at or near the possible maximum in the periods of free respiration. In this case asphyxia could not cause a demonstrable increase. Although it is easy to show that the rate of liberation under the same conditions can be readily increased through the secretory nerves, namely, by electrical stimulation of the splanchnic, some experiments were made in which the use of a chemical anesthetic was avoided, naturally by the substitution of methods which rendered the animal completely insensitive. No difference in the result was found.

Experiment 3. Condensed protocol. Dog (male). Weight, 11.05 kgm. Rendered insensitive by destruction of the cerebral cortex with some of the underlying centrum ovale. Under ether, trephined and curetted away the cerebral cortex. Tracheal and jugular cannulae inserted and specimen of jugular blood obtained. "Short" cava pocket made, renal, coeliac and mesenteric arteries and abdominal aorta being tied. Started artificial respiration, although the animal was breathing spontaneously, and collected the following adrenal blood samples:

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	grams	*minutes*	*seconds*	*grams*	
1	7.0	1	45	4.0	Without asphyxia
2	7.7	2	35	3.0	Without asphyxia
3	8.5	4		2.1	During asphyxia
4	8.8	5	40	1.5	During asphyxia
5	4.5	6		0.75	Without asphyxia

~A sixth specimen was collected during asphyxia, but partial clotting in the cannula prevented proper estimation of the rate of flow.

Specimens of the uterus tracings from experiment 3 are reproduced in figure 6. A regular increase in the effect is observed from the second to the sixth adrenal specimen, without any apparent relation to the presence or absence of asphyxia. The second specimen (observation 28) caused a smaller increase
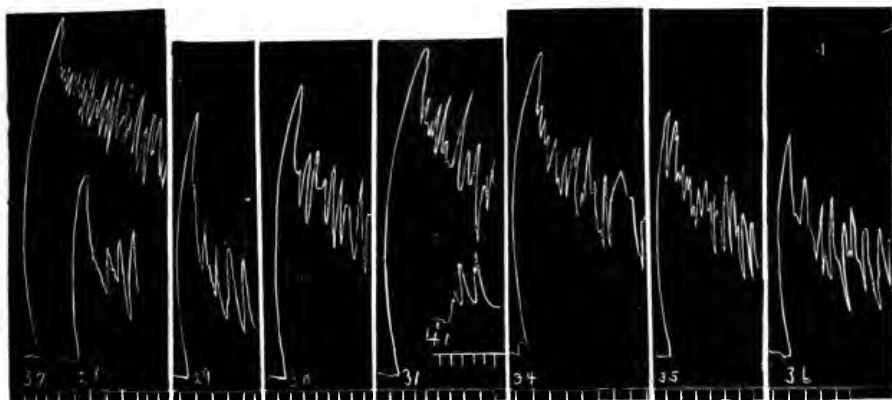
FIG. 6. UTERUS TRACINGS. BLOOD FROM DOG WITH CEREBRAL HEMISPHERES DESTROYED

At *28* Ringer was replaced by the second adrenal specimen (without asphyxia); at *29* by the third adrenal specimen (asphyxia); at *30* by the fourth adrenal specimen (asphyxia); at *31* by the fifth adrenal specimen (without asphyxia); at *37* by the sixth adrenal specimen (asphyxia); at *41* by jugular vein blood. All bloods diluted with fifteen volumes Ringer. At *34* adrenalin in jugular blood (1:2,000,000); at *35* adrenalin in jugular blood (1:3,000,000); at *36* adrenalin in jugular blood (1:4,000,000) replaced Ringer. The adrenalin bloods, after being made up to the concentrations mentioned, were diluted with 15 volumes Ringer before application to the segment. (Reduced to one-half.)

of tone in the segment than the third (observation 29). But this was not due to its having been collected during asphyxia, for the fifth specimen (observation 31), collected without asphyxia, produced a decidedly greater effect than the fourth (observation 30), collected during asphyxia. The third and fourth specimens were both collected during asphyxia; but the fourth caused the greater effect upon the uterus, particularly as regards

the persistence of the increased tone. The somewhat greater concentration of epinephrin in the fourth specimen, as compared with the third, cannot be connected with any stimulation of the epinephrin secretion by asphyxia, since this would equally be present during collection of the third specimen. It is undoubtedly due to the fact that the rate of flow when the fourth specimen was being obtained was somewhat less than when the third specimen was being obtained. The rate of liberation of epinephrin per minute being approximately constant at this time, a diminution in the rate of blood flow was necessarily associated with a corresponding increase in the epinephrin concentration. An increase in concentration from 1:4,000,000 to 1:3,000,000, or from 1:3,000,000 to 1:2,000,000 (observations 34 to 36) could easily be detected by the uterus segment, and assuredly much smaller changes in concentration.

Elliott (4) states that after brain mutilations in cats the epinephrin store of the adrenals is markedly diminished through the nerves coming to the semilunar ganglion from the sympathetic. He considers that this is due to irritation of a secretory nerve path caused by the brain lesion leading to increased discharge of epinephrin. However, he made no experiments to show that the rate of liberation is, as a matter of fact, increased. Assays of adrenal blood specimens from the dog studied in experiment 3 showed, indeed, that the output of epinephrin per kilogram of body weight per minute was less than that usually found in anesthetized dogs with intact central nervous system. The lack of the stimulating action on the secretory mechanism attributed by various writers to anesthetics might seem to afford an explanation. There is no real proof, however, that anesthetics possess such an action. In any case, the moderate rate of output of epinephrin with free respiration ought to have supplied the most favorable condition for bringing out an asphyxial increase, if any decided increase could be produced by asphyxia.

In the next experiment (experiment 4), mutilation of the brain and chemical anesthesia were both avoided by rendering the animal insensitive through increase of intracranial pressure.

Experiment 4. Condensed protocol. Dog (female). Weight, 10.4 kgm. Under ether, trephined; inserted rubber bag (condom) through trephine hole; brought up the pressure to 250 mm. of mercury, and kept the pressure between 200 and 250 mm. through the whole time of collection of the adrenal bloods. Ether was discontinued as soon as the pressure was begun, and artificial respiration was started. A sample of jugular blood was obtained. Then a "short" cava pocket was made, the renal, coeliac and mesenteric arteries and abdominal aorta being tied. The following specimens of adrenal blood were collected:

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	grams	*minutes*	*seconds*	*grams*	
1	6.3	1	10	6.0	Without asphyxia
2	6.8	4	15	1.6	With asphyxia
3	4.5	3	20	1.4	Without asphyxia
4	5.7	3	40	1.6	Without asphyxia
5	6.8	4		1.7	During asphyxia
6	9.0	5	30	1.7	During asphyxia
7	7.7	3		2.6	Without asphyxia
8	13.8	5	20	2.6	Without asphyxia
9	9.0	5		1.8	With greater degree of asphyxia than before
10	6.4	2	40	2.5	Without asphyxia

Combined weight of adrenals, 1.46 grams. Clot was removed from the cannula between collection of the fourth and fifth samples, and again between the ninth and tenth samples.

In figures 7 and 8 are reproduced some of the intestine segment tracings from experiment 4. Figure 7 shows that the third adrenal specimen (obtained without asphyxia) had a somewhat greater inhibitory power than the second specimen (collected during asphyxia), corresponding to the somewhat slower flow of the third specimen. The difference in the flows, however, was so slight that asphyxial stimulation of the secretion ought to have easily produced an excess of epinephrin in the second specimen had any detectable stimulation existed. The concentration of epinephrin in the third specimen was about $1:3,000,000$ (fig. 8, observations 40 and 44), and much greater than $1:5,000,000$ (observation 42). Similar comparisons of the

other samples collected with free respiration and during asphyxia yielded the same result. In no case was any difference found which could be attributed to asphyxia. This was not because the concentrations were already maximal, although owing to the relatively small blood flow they were fairly high for a dog.

The objection might be offered that the high intracranial pressure had rendered the central mechanism concerned in the
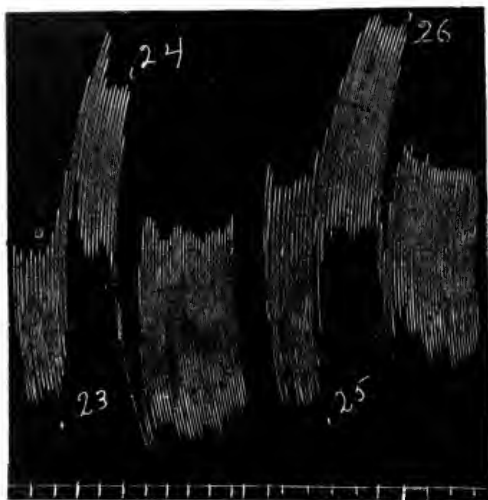
FIG. 7. INTESTINE TRACINGS. BLOOD FROM DOG RENDERED INSENSITIVE BY INCREASED INTRACRANIAL PRESSURE (EXPERIMENT 4)

At *23* Ringer was replaced by jugular blood, and this at *24* by the third adrenal specimen (collected without asphyxia). At *25* Ringer was replaced by jugular blood, and this at *26* by the second adrenal specimen (asphyxia). Bloods diluted with two volumes Ringer. (Reduced to two-thirds.)

liberation of epinephrin anemic, supposing it to be situated within the skull, and that it was therefore incapable of responding to the asphyxial blood.[2] We have shown, however, that

[2] It was, indeed, with the object of testing this idea that the high intracranial pressure was maintained throughout the experiment. Two of the adrenal blood samples, the fifth and the eighth, had a decidedly smaller concentration of epinephrin, as tested by rabbit intestine segments, than samples obtained earlier, as well as later in the series. We have never observed this phenomenon except

(in the cat) a central mechanism for epinephrin secretion exists in the upper part of the thoracic cord (5); and there is no apparent reason why this should not have been stimulated by asphyxia. Further, it must be distinctly pointed out that even if it were clearly proved that a "center" exists in the bulb or higher, the integrity of which is essential to the reduction of the epi-
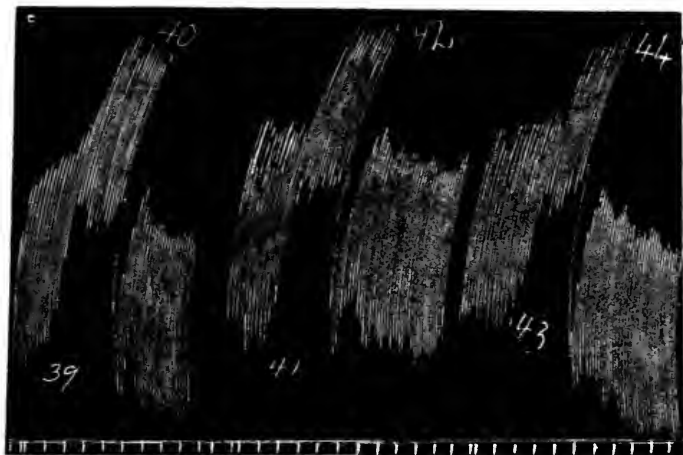
FIG. 8. INTESTINE TRACINGS. BLOOD FROM THE SAME DOG USED FOR FIGURE 7

At *39* Ringer was replaced by jugular blood and this at *40* by the third adrenal blood specimen (without asphyxia). At *41* Ringer was replaced by jugular blood, and this at *42* by adrenalin (1: 5,000,000) in jugular blood. At *43* Ringer was replaced by jugular blood and this at *44* by adrenalin (1: 3,000,000) in jugular blood. The bloods were diluted with two volumes Ringer, the adrenalin bloods being first made up in undiluted blood to the concentrations mentioned, and the mixture then diluted (with two volumes Ringer). (Reduced to two-thirds.)

nephrin store of the adrenals under various conditions (Elliott (4)), this is no direct proof that such a center controls the rate of

in two dogs with increased intracranial pressure. In the cats with increased intracranial pressure it was not seen. In any case, in the experiment under discussion the deficiency of epinephrin in these two samples affords no evidence that asphyxia stimulates the secretion of epinephrin. For one of the specimens (the fifth) was collected during asphyxia and the other (the eighth) with free respiration. It is conceivable, of course, that an intracranial "centre" already crippled by anaemia, might have its paralysis completed by a period of asphyxia.

liberation of epinephrin into the blood. For an increased output of epinephrin is not the only way in which a diminution of the epinephrin store in the adrenals could be caused. Nevertheless, in experiment 5 an attempt was made to take account of this objection by lowering the intracranial pressure as soon as the animal became insensitive. The result of Experiment 5, however, differed in no essential way from that of Experiment 4.

Experiment 5. Condensed protocol. Dog (male). Weight, 9.25 kgm. Rendered insensitive by increased intracranial pressure. Under ether, trephined and inserted rubber bag. Got up pressure to 250 mm. of mercury. Discontinued ether. Decreased the pressure as the blood pressure fell. Started artificial respiration. Obtained specimen of jugular blood. Made short cava pocket, tying renal, coeliac and mesenteric arteries and abdominal aorta. Collected the following specimens of adrenal blood:

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	grams	*minutes*	*seconds*	*grams*	
1	14.3	1		14.3	Without asphyxia
2	8.7		45	11.6	Without asphyxia
3	8.8	1	50	5.0	During asphyxia
4	6.7	1	40	4.2	During asphyxia
5	14.9	2	15	6.6	Without asphyxia
6	12.4	2	30	5.0	Without asphyxia
7	20.7	2	55	6.8	During asphyxia
8	10.3	1	30	6.8	Without asphyxia

Another specimen of jugular blood was now obtained. All the bloods were centrifuged, and the sera tested on rabbit intestine and uterus segments. Combined weight of adrenals, 1.32 grams.

Specimens of intestine segment tracings from experiment 5 are reproduced in figures 9 and 10. In figure 9, it is shown that serum of the sixth adrenal specimen (observation 21) caused practically the same inhibition as serum of the seventh specimen (observation 23), although the seventh was collected during asphyxia, and the sixth with free respiration. Adrenalin assays (samples of the tracings are reproduced in fig. 10), proved that

the sera of the sixth and seventh specimens contained much more than 1 : 8,000,000 epinephrin (observation 29), and as nearly as possible 1 : 5,000,000 (observation 27), corresponding to an output of less than 0.0001 mgm. per kilo of body weight per minute.   This is below rather than above the average output for dogs anesthetized with ether, and the concentration is considerably below the average for the sera, as estimated in this way.
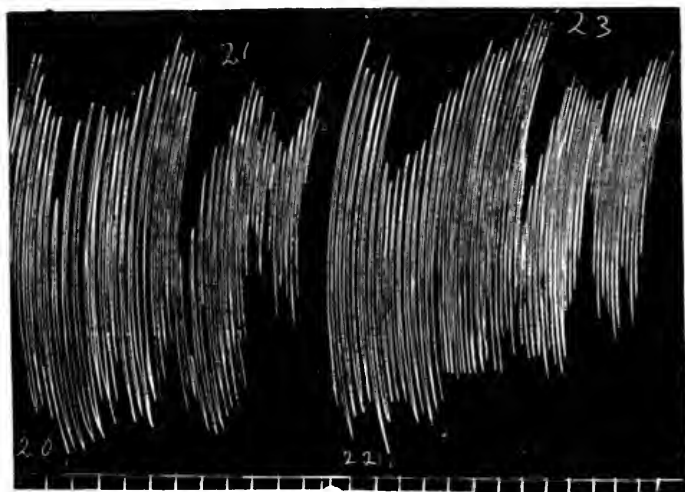
Fig. 9. Intestine Tracings. Blood Serum from Dog Rendered Insensitive by Increased Intracranial Pressure (Experiment 5)

At 20 Ringer was replaced by jugular blood serum, and this at 21 by serum of the sixth adrenal specimen (without asphyxia). At 22 Ringer was replaced by jugular serum, and this at 23 by the serum of the seventh adrenal specimen (asphyxia). All the sera were diluted with two volumes Ringer. (Reduced to two-thirds.)

The concentration of epinephrin in those sera is much below the possible maximum. Therefore, it ought to have been easy to detect an increase due to asphyxia, had asphyxia been capable of producing a great and abrupt augmentation in the output.

Some of the uterus tracings are reproduced in figure 11. The fifth adrenal serum, collected without asphyxia, caused a somewhat greater increase of tone than the fourth, collected during

asphyxia, despite the fact that the blood flow for the fifth was rather greater than for the fourth specimen; and that the concentration in the fifth specimen might therefore have been expected to be a little less than in the fourth. The difference between the two specimens was confirmed by observations 43 and 45, made with a greater degree of dilution. With a smaller dilution, the increase of tone was the same for the two speci-
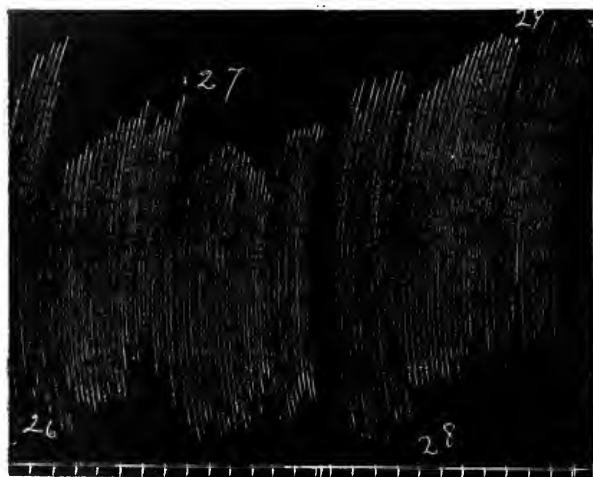
FIG. 10. INTESTINE TRACINGS. SERA FROM SAME DOG USED FOR FIGURE 9

At *26* Ringer was replaced by jugular serum, and this at *27* by adrenalin (1:5,000,000) in jugular serum. At *28* Ringer was replaced by jugular serum and this at *29* by adrenalin (1:8,000,000) in jugular serum. The adrenalin sera, after being made up to the concentrations mentioned, were diluted with two volumes Ringer before application to the segment. (Reduced to two-thirds.)

mens (observations 46 and 47), this being approximately the maximal increase of which the segment was capable in response to the combined serum and epinephrin effects of these sera. Observations 32 and 33 (on another uterus segment) gave practically the same effect for the sera of the sixth and seventh adrenal specimens, when the persistence of the increase of tone is taken into account, the uterus response being almost maximal. In a greater dilution, however, a difference was brought out

(observations 35 and 36) in favor of the seventh specimen, collected during asphyxia. The intestine tracings showed that any difference which existed between these two specimens must have been slight. It must be remarked here that in comparing uterus tracings the absolute difference in height has not the
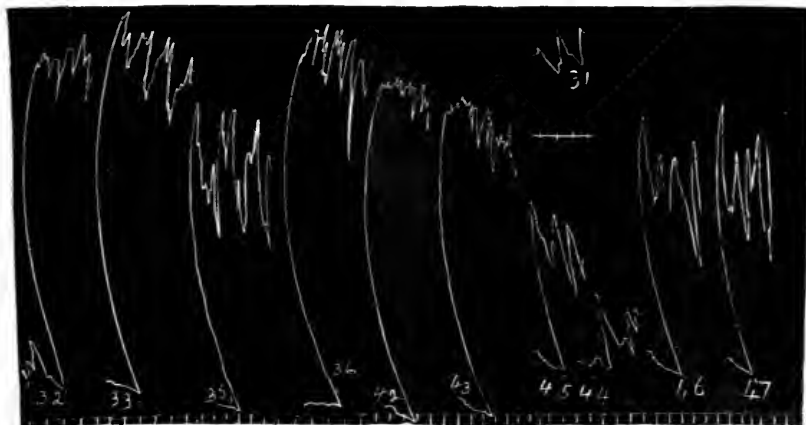
FIG. 11. UTERUS TRACINGS. SERA OF SAME DOG USED FOR FIGURES 9 AND 10

At 31 Ringer was replaced by jugular serum; at 32 by serum of sixth adrenal specimen (collected without asphyxia); at 33 by serum of seventh adrenal specimen (asphyxia). The three sera were diluted with four volumes Ringer. At 35 Ringer was replaced by serum of the sixth adrenal blood specimen; at 36 by serum of the seventh specimen, in each case diluted with six volumes Ringer. At 42 Ringer was replaced by serum of the fifth adrenal specimen (without asphyxia); at 43 by serum of the fourth adrenal specimen (asphyxia). The sera were diluted with four volumes Ringer. At 44 Ringer was replaced by serum of the fourth adrenal specimen; at 45 by serum of the fifth specimen, in each case diluted with six volumes Ringer. At 46 Ringer was replaced by serum of the fourth adrenal specimen; at 47 by serum of the fifth specimen, each diluted with three volumes Ringer. Observations 31 to 36, inclusive, were made on one uterus segment; observations 42 and 43 on another segment of the same uterus; observations 44 to 47 on a segment of another uterus. (Reduced to one-half.)

same quantitative value as the difference in the amount of inhibition of the intestine segments. All that can be deduced from observations 35 and 36 is that the combined serum and epinephrin effect of the seventh specimen is greater than that of the sixth. No estimate can be formed from these observations

as to the amount of the difference.   A sensitive uterus segment practically always gives a somewhat larger effect for a later than for an earlier specimen in the absence of asphyxia, and the asphyxial periods in this experiment do not seem to have sensibly modified this progression.   The blood flows did not vary much from the fifth to the eighth specimens.

A similar experiment to experiment 4 was performed on a cat (experiment 6), and with a similar negative result as regards any influence of asphyxia upon the epinephrin output.

Experiment 6.   Condensed protocol.   Cat (female).   Weight, 2.035 kgm.   Rendered insensitive by increased intracranial pressure.

10.35 a.m.   Under ether, trephined, and inserted pressure bag.   Got up pressure to 250 mm., and discontinued ether.

10.45 a.m.   Inserted tracheal and jugular cannulae.   Obtained jugular blood.

11.05 a.m.   Short cava pocket made, the renal, coeliac and mesenteric arteries and abdominal aorta being tied.

11.10 a.m.   Started artificial respiration, although the cat was breathing well spontaneously.

11.15 a.m.   Pressure 250 mm.   Eye reflexes just gone.   Some voluntary respirations.   Pulse, 175.   Lowered pressure to 170 mm.

11.17 a.m.   Lowered pressure to 130 mm.   Gasping respirations and tongue movements.

11.18 a.m.   Pressure raised to 230 mm.

11.20 a.m.   Started collection from cava pocket.

11.21 a.m. to 11.24 a.m.   Gasping respirations.   Pulse, 156.   Pressure raised to 260 mm. and maintained above 200 mm. during collection of the following adrenal blood specimens.

NUMBER OF ADRENAL SPECIMEN	BLOOD COLLECTED	TIME OF COLLECTION		BLOOD FLOW PER MINUTE	REMARKS
	grams	*minutes*	*seconds*	*grams*	
1	2.5	1		2.5	Without asphyxia
2	4.5	2	.	2.2	Without asphyxia
3	3.6	2	45	1.3	Without asphyxia
4	2.5	3	30	0.7	During asphyxia
5	1.7	4		0.4	Without asphyxia
6	2.0	6	30	0.3	Without asphyxia

Spontaneous respirations were present during collection of the second and third specimens. No eye reflexes were present during collection of the adrenal samples. Blood was obtained at the end from the abdominal aorta. Combined weight of adrenals, 0.338 gram.

Some of the intestine tracings are reproduced in figures 12 and 13. The third specimen (observation 17), collected without
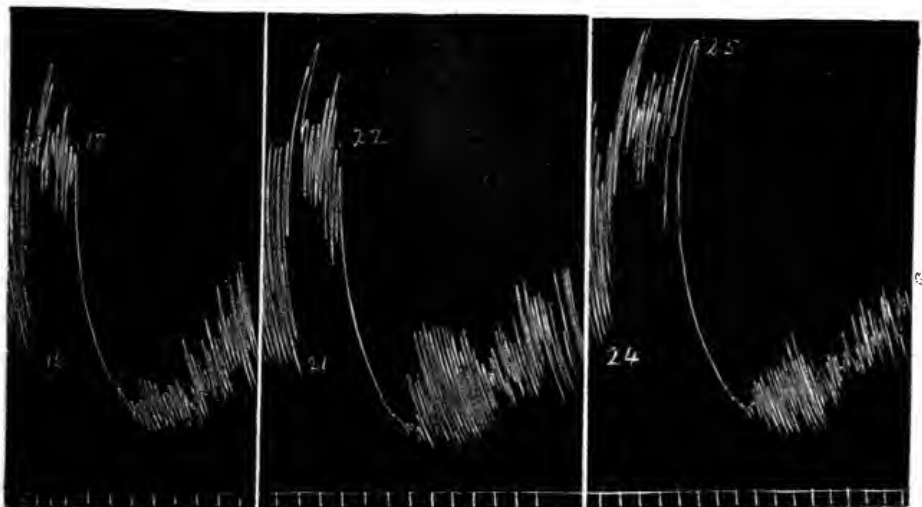
FIG. 12. INTESTINE TRACINGS. BLOOD FROM CAT RENDERED INSENSITIVE BY INCREASED INTRACRANIAL PRESSURE

At *16* Ringer was replaced by jugular blood and this at *17* by the third adrenal specimen (collected without asphyxia). At *21* Ringer was replaced by jugular blood and this at *22* by the fourth adrenal specimen (asphyxia). At *24* Ringer was replaced by jugular blood and this at *25* by the fifth adrenal specimen (without asphyxia). Bloods diluted with eight volumes Ringer. (Reduced to one-half.)

asphyxia, certainly produces a somewhat smaller effect upon the intestine segment (the inhibition is sooner recovered from) than the fourth specimen (observation 22), collected during asphyxia. But this is clearly associated with the greater flow during collection of the third specimen, and the fourth adrenal specimen is no richer in epinephrin than the fifth specimen (observation 25), collected without asphyxia. The concentration in the

fourth specimen is really less than in the fifth. With a greater
degree of dilution (fig. 13), the greater effect of the fifth speci-
men than of the fourth in inhibiting the intestine segment be-
came more evident. The slightly inferior inhibitory power of
the third specimen as compared with the fourth is thus seen to
have no demonstrable relation to the presence or absence of
asphyxia. The adrenalin assay showed that the fifth specimen
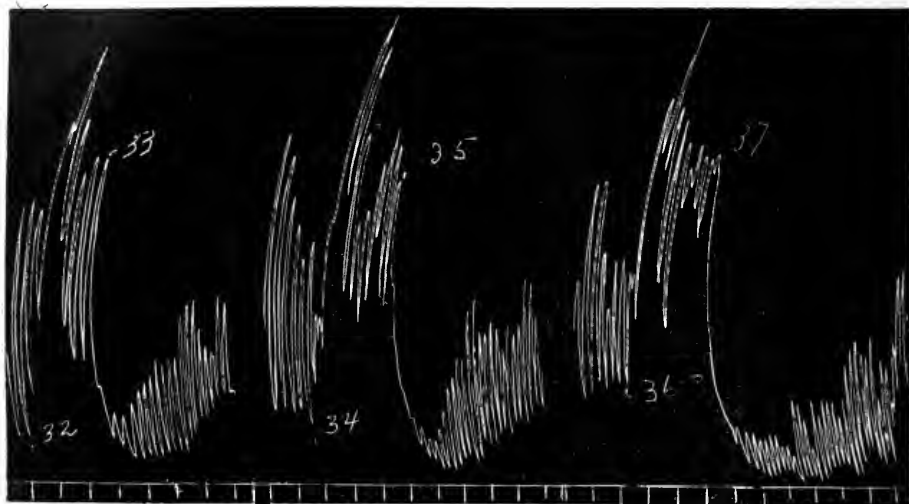
Fig. 13. Intestine Tracings. Blood from Same Cat Used for Figure 12

At *32* Ringer was replaced by jugular blood and this at *33* by the third adrenal
specimen (without asphyxia). At *34* Ringer was replaced by jugular blood and
this at *35* by the fourth adrenal specimen (asphyxia). At *36* Ringer was replaced
by jugular blood and this at *37* by the fifth adrenal specimen (without asphyxia).
Bloods diluted with sixteen volumes Ringer. (Reduced to two-thirds.)

had a smaller concentration than 1:800,000, and a greater
concentration than 1:1,100,000. This is as great a concen-
traction as is ever found in the blood of the cat, collected and
tested in this way.

The uterus tests (samples of the tracings are reproduced in
figure 14) confirmed the results of the assay on the intestine
segments. Observations 48, 50 and 51 show that the tone-
increasing power of the fifth adrenal specimen was intermediate

between that of indifferent blood containing 1:1,600,000 adrenalin and that of indifferent blood containing 1:800,000 adrenalin. The third specimen (without asphyxia) produced only a slightly smaller increase of tone than the fourth specimen (collected during asphyxia). The second specimen produced a much smaller effect than any of the succeeding samples, corresponding to its greater rate of flow.

FIG. 14. UTERUS TRACINGS.   BLOOD FROM SAME CAT USED FOR FIGURES 12 AND 13

Ringer was replaced at *45* by the second adrenal specimen (without asphyxia); at *46* by the fourth specimen (asphyxia); at *47* by the third specimen (without asphyxia); at *48* by the fifth specimen (without asphyxia).   Bloods diluted with sixteen volumes Ringer.   Ringer was replaced at *50* by adrenalin in jugular blood (1:1,600,000); at *51* by adrenalin in jugular blood (1:800,000).   The adrenalin was added to the undiluted bloods to the concentrations mentioned, the mixtures being then diluted with 16 volumes Ringer before application to the segment.   (Reduced to one-half.)

The output of epinephrin per minute, assuming that the fifth sample had a concentration of 1:1,000,000, would be 0.0004 mgm. (0.0002 mgm. per kilo of animal per minute), which is about the same as is found in cats under the experimental conditions, but anesthetized with urethane without increased intracranial pressure (6).   In this experiment, accordingly, neither the absence of a chemical anesthetic nor the increased intracranial pressure seems to have diminished the output.

This conclusion is supported by experiment 7, a control experiment in which urethane was combined with increased intracranial pressure.

Experiment 7. Condensed Protocol. Cat. Weight 2.425 kgm.

9.20 a.m.   5 grams urethane.

10.00 a.m.   Tracheal and jugular cannulae inserted and jugular blood obtained. Trephined and inserted pressure bag. "Short" cava pocket made, renal, coeliac and mesenteric arteries and abdominal aorta being tied. The following samples of adrenal blood were now obtained, with no pressure in the intracranial bag.

1st sample: 0.9 gram in 20 seconds (2.7 grams per minute).

2nd sample: 1.9 grams in 1 minute, 30 seconds (1.3 grams per minute).

Pressure in the bag was now got up, to 250 mm. of mercury, and the following samples of adrenal blood collected:

3d sample: 2.6 grams in 2 minutes, 10 seconds (1.3 grams per minute).

4th sample: 4.3 grams in 3 minutes, 20 seconds (1.3 grams per minute).

5th sample: 3.3 grams in 3 minutes (1.1 grams per minute).

6th sample: 4.0 grams in 4 minutes (1.0 gram per minute).

Combined weight of adrenals 0.390 gram.

The adrenalin assay showed that the second adrenal blood specimen contained approximately 1 : 2,000,000 epinephrin, corresponding to an output per minute for the animal of 0.00065 mgm., or 0.00025 mgm. per kilogram of body weight per minute, an output within the range observed in cats anaesthetised with urethane, without increased intracranial pressure.[3] A comparison of the epinephrin concentration in specimens of the adrenal blood collected without and with increased intracranial pressure revealed no difference in the rate of output. Thus,

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[3] See table 2, Journ. Pharm. and Exp. Therap., 1917, x, 4.

the second specimen caused an inhibition of the intestine segment only slightly less than that caused by the sixth specimen, corresponding to the somewhat greater blood flow when the second was being collected.

Since the tests instituted on adrenal blood directly collected from the cava pocket had failed to yield unequivocal evidence of an increase in the rate of liberation of epinephrin associated with asphyxia, it did not seem probable that observations on the highly diluted adrenal blood obtained from the inferior cava central to the orifices of the adrenal veins (7) would reveal a difference. Nevertheless, the catheter method was tried, but again with a negative result. In one experiment, the condensed protocol of which is published elsewhere (8), a comparison of cava blood drawn off by a catheter from above the level of the adrenals during asphyxia was made with blood similarly obtained during stimulation of the sciatic nerve, and with blood collected through the catheter in the absence both of asphyxia and sensory stimulation. All these bloods behaved in the same way towards rabbit intestine and uterus segments. There was also no distinct difference between the action of any of them, and that of indifferent blood collected from the lower part of the inferior cava. It was proved that the reason for this was the dilution of the adrenal contribution by indifferent cava blood. The degree of dilution with indifferent blood necessary to render undetectable pure adrenal blood collected from the cava pocket, and containing a content of epinephrin within the ordinary range was ascertained. It was shown to fall within the limits of the dilution which the adrenal blood must normally undergo in the cava. We do not see how it is possible to make quantitative comparisons of the rate of output of epinephrin under different conditions by the catheter method.

CONCLUSION

An attempt was made to determine whether asphyxia produces a detectable increase in the rate of liberation of epinephrin from the adrenals, as determined by testing adrenal vein blood on rabbit intestine and uterus segments. The result was negative.

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PHARMACOLOGICAL STUDIES OF THE IPECAC ALKALOIDS AND SOME SYNTHETIC DERIVATIVES OF CEPHAELINE[1]

I. STUDIES ON TOXICITY

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Three alkaloids have been obtained from ipecac, the root of either Cephaelis Ipecacuanha or Cephaelis acuminata. These are emetine, cephaeline and psychotrine. While it is claimed that other alkaloids have been isolated from ipecac, these findings have not been confirmed by subsequent investigations.

From an analysis of 145 different lots and samples of ipecac representing in all many thousand pounds of drug, our records show that commercial ipecac averages 2.17 per cent of total alkaloids. The lowest assay was 1.83 and the highest 2.84 per cent. The average amount of emetine is to that of cephaeline as 63 is to 37. There is little or no difference in alkaloidal strength between Rio and Carthagena ipecac and the partition of the emetine and cephaeline in the two species is in general the same. These figures are not entirely in harmony with those usually published which, however, were based on very few assays and were no doubt due to individual variations in the drug assayed.

Carr and Pyman have assigned to psychotrine the formula $C_{28}H_{36}N_2O_4$, to cephaeline $C_{28}H_{38}N_2O_4$, and to emetine $C_{29}H_{40}N_2O_4$. These formulas show the close chemical relationship between these alkaloids. Cephaeline is psychotrine plus H_2, and emetine

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[1] Other papers appearing in this series will be as follows: Studies on Emetic Effect and Irritant Action; Studies on Protozoacidal and Bactericidal Action; Studies of the Effect on Circulation, Respiration and Secretion.

is cephaeline plus CH_2, or methyl-cephaeline. Emetine and cephaeline produce similar pharmacological effects qualitatively but differ considerably in the intensity of their action. Both are bactericidal and amebacidal, and are irritant, emetic and toxic in small doses. They differ, however, in that emetine is stronger as an amebacide and protozoacide and is weaker as an irritant, emetic or toxic agent.

Since methylating cephaeline to produce emetine is known to increase the amebacidal property and diminish the emetic, irritant and toxic effects, it was thought probable that ethers of cephaeline with the higher homologous alcohols might further enhance the therapeutic value of this alkaloid. Accordingly the following new ethers of cephaeline were prepared,[2]—ethyl, propyl, iso-propyl, butyl, iso-butyl, tertiary-butyl, amyl, iso-amyl, and allyl. The hydrochlorides, hydrobromides, phosphates, sulphates and hydroiodides of the majority of these were obtained. These cephaeline compounds were made by boiling cephaeline with sodium ethylate and the corresponding alkyl bromide or iodide, extracting the cephaeline ether, thus formed, with ether; concentrating the ether solution, acidifying and allowing the salt to crystallize, and purifying by recrystallization. The hydrobromides of this series are slightly soluble in cold water and readily soluble in hot water. The hydrochlorides are more soluble than the hydrobromides. The phosphates and sulphates are the most readily soluble and the hydroiodides are the least soluble.

Most of the pharmacological work of early investigators on the ipecac alkaloids was carried out with mixtures of emetine and cephaeline, rather than the pure alkaloids, and hence their quantitative results may be questioned. The alkaloids used in our experiments were prepared in the Chemical Research Laboratory and their identity and purity established.

[2] Prepared by J. W. Meader, Chemical Research Laboratory, Eli Lilly and Company.

TOXICITY

In making the toxicity tests, the main purpose was to determine the relative killing power of these various derivatives of cephaeline. The animals used were white rats, guinea-pigs and rabbits. The following table gives a summary of the results obtained, the doses being gram of alkaloidal salt per kilogram of animal

DRUG	SUBCUTANE-OUSLY WHITE RATS	SUBCUTANE-OUSLY GUINEA PIGS	INTRAVEN-OUSLY RABBITS
Psychotrine hydrochloride................	1.00+	0.2+	
Cephaeline hydrochloride................	0.0065	0.008	
Cephaeline methyl ether (emetine) HCl..	0.012	0.016	0.005
Cephaeline ethyl ether HBr..............	0.015		
Cephaeline propyl ether phosphate.......	0.045		0.007
Cephaeline iso-propyl ether HCl.........	0.045	0.050	
Cephaeline n-butyl ether HCl............	0.025		
Cephaeline iso-butyl ether HCl..........	0.030		
Cephaeline tertiary-butyl ether HBr.....	0.030		
Cephaeline iso-amyl ether HBr..........	0.060		0.010
Cephaeline allyl ether HBr..............	0.020		

The most striking fact brought out by these figures is the decrease in toxicity as the higher alcohols are combined with cephaeline. The addition of a methyl group to cephaeline to make cephaeline methyl ether (emetine) has decreased the toxicity one-half, and by replacing this methyl group by an iso-amyl group the toxicity is only one-fifth that of the cephaeline methyl ether hydrochloride or about one-tenth as toxic as cephaeline hydrochloride itself.

Another remarkable feature, considering the close chemical relation, is the very low toxicity of psychotrine. Even when given in a dose as large as 1 gram per kilogram of animal this alkaloid was not fatal to white rats. The minimum lethal dose for this substance was not determined. Smaller lethal doses have been published, but these may have been due to a contamination of the material as it is rather difficult to obtain psychotrine free from the other alkaloids of ipecac.

It will also be noted that the difference in toxicity between emetine hydrochloride and cephaeline propyl ether phosphate

or the isoamyl ether hydrochloride when given intravenously to rabbits is not nearly so great as when these substances are given subcutaneously to rats or guinea-pigs. When given subcutaneously emetine hydrochloride is five times as toxic as cephaeline isoamyl ether hydrobromide, but when given intravenously it is not more than twice as toxic. This is probably due in part to the difference in their rate of absorption from the subcutaneous tissues as other experiments to be described later will tend to prove.

It is noteworthy that the cephaeline compounds containing the higher alcohol radicals decrease in toxicity, reaching the lowest degree of toxicity in cephaeline iso-amyl ether. It is also to be remarked that all the cephaeline derivatives kill slowly. In the above experiments the animals were observed for at least seven days, and a death within that time was taken into account in determining the lethality. The minimum lethal dose (M.L.D.) usually killed in two to four days. The following protocol will illustrate the method:

Cephaeline propyl ether phosphate

WEIGHT OF RAT	DOSE PER KILO-GRAM	INJECTED	RESULT	REMARKS
gm.				
174	0.035	11–12–1915	Survived	Observed 10 days
123	0.035	11–20–1915	Survived	Observed 7 days
141	0.035	10–28–1915	Died	On tenth day
186	0.040	11–12–1915	Survived	Observed 10 days
166	0.040	11–20–1915	Survived	Observed 10 days
110	0.040	10–30–1915	Died	On ninth day
140	0.040	12– 3–1915	Died	On eighth day
140	0.040	12– 3–1915	Survived	Observed 10 days
139	0.045	11– 2–1915	Died	On second day
194	0.045	11–12–1915	Died	On third day
176	0.050	11–12–1915	Died	On second day
119	0.050	11– 4–1915	Died	On fourth day

0.0075 gram in each cubic centimeter.
Minimal Lethal Dose = 0.045 gram per kilogram.
Injected into subcutaneous tissues of abdomen.

The subject of the cumulative poisonous action of emetine has been suggested and some work along this line has been done

by Dale (1).   We have found that one-half the minimal lethal dose, repeated at daily intervals subcutaneously, is quite uniformly fatal when a dose totaling two and one-half times the minimal lethal dose has been given.   Thus the minimal lethal dose of emetine hydrochloride for rabbits is 10 mgm. per kilogram when given in a single subcutaneous injection; whereas 5 mgm. per kilogram injected daily will kill after the fifth dose or a total of 25 mgm. per kilogram.   Cephaeline iso-amyl ether hydrochloride, which is one-fifth as toxic as emetine hydrochloride when given subcutaneously in a single injection, bears this same relation when given in comparable sublethal dose at daily intervals.   Thus four daily injections of 30 mgm. per kilogram, or five daily injections of 25 mgm. per kilogram each, cause death of the animal.   Giving cephaeline iso-amyl ether hydrochloride in daily injection of 5 mgm. per kilogram the rabbit succumbs as a rule after the twenty-second dose; that is, after a total of 120 mgm., which again is five times the fatal dose of emetine hydrochloride when given in daily injections of 5 mgm. per kilogram. These toxicity figures would indicate then that the cephaeline derivatives when given in small daily doses, one-tenth to one-twelfth their minimal lethal dose, will eventually prove toxic and that the period necessary to produce death in rabbits is about three weeks.   Smaller doses than these may prove fatal over longer periods of time, but no experiments to demonstrate this were made.

It is evident that the animals of different genera do not succumb to exactly the same dose of these alkaloids, and it is clearly impossible to make an exact analogy between these toxic doses and those for man.   No authentic case of fatal poisoning in man from a single dose of emetine has been recorded   There has, however, been a fatality reported by Rowntree and Levy (2) in which emetine hydrochloride was administered daily for nineteen days to a man weighing 69.5 kgm.   The total amount given was 1.74 grams, an average daily dose of 90 mgm. or 1.3 mgm. per kilogram.   This corresponds closely with the tox c dose of emetine for rabbits, which we find to be 1 mgm. per kilogram given daily for about three weeks.   These figures give

some basis for estimating a single toxic dose for a man of 70 kgm. which then, by analogy, would be 0.7 gram or 10.8 grains.

Another death from emetine injections is reported by Dr. J. A. Snell (3). The patient was a girl, 5 years old who received 10.6 grains (688 mgm.) in twenty-one days, an average of 33 mgm. per day. Assuming the child's weight to have been 17 kgm., the fatal daily dose was 2 mgm. per kilogram.

Capt. Howard H. Johnson and Lieut. John A. Murphy (4) report two deaths in their service as probably due to emetine. One patient, a man of 22 years, received emetine as follows: August 10 to 19, 9 grains; August 21 to 24, 3 grains; September 5 to 15, 10 grains; September 27 to October 3, 3 grains; a total of 25 grains in 44 days. The second, a man of 38 years, received 1 grain of emetine a day for twelve and one-half days and then, after an interval of five days, was given ½ grain daily for fourteen days followed by 1 grain daily for four days, making a total of 23½ grains in thirty-five days. The body weight is not given in either case. An unexpected finding in these two cases at necropsy was the absence of inflammation of the alimentary tract which in fact was normal in appearance throughout its extent.

In this connection it may be of interest to review some of the published cases in which large doses of emetine have not been fatal. Johnson and Murphy (loc. cit.) report five cases showing toxic manifestations attributable to large doses of emetine. These doses in the five cases were as follows; 18 grains in twenty-six days, 29 grains in thirty-four days, 25⅔ grains in twenty-eight days, 22 grains in twenty-two days, and 22 grains, over a period not stated, to a patient who at some previous time had received a course of 23 grains. All these cases showed lassitude, weakness and extreme exhaustion. Neuritis, muscular tremor, rapid pulse and diarrhea were present in nearly all. Spehl and Collard (5) report the case of a man, age 28, who received 60 mgm. daily for six days, and then 90 mgm. a day for six days longer, making a total of 900 mgm. in a period of twelve days, or 75 mgm. per day. Assuming that this man weighed 65 kilograms he received somewhat more than 1 mgm.

per kilogram daily.   Severe symptoms followed the above course of treatment but the patient gradually recovered during two weeks from date of onset.   Eshleman (6) reports the case of a man who received 1 grain, twice a day for five or six days.   This patient developed a severe peripheral neuritis and a purpuric eruption of the skin.   Allan (7) reports having given 2.4 grains of emetine hydrochloride followed on the succeeding day by an injection of 4 grains.   The patient became nauseated and vomited once, four and a half hours after this injection.   No other ill effects are mentioned.   This is the largest single subcutaneous dose we have found recorded.   Lyons (8) reports a man having taken $1\frac{1}{3}$ grains a day for sixteen days, a total of $21\frac{1}{3}$ grains.   This patient showed general muscular weakness and a mild peripheral neuritis.   There are many case reports where 1 grain of emetine has been given daily for twelve days with no ill effects.

DISCUSSION

From these experiments and case reports it would seem that the toxicity of emetine has generally been over-stated.   The toxic dose of 10 grains is certainly a rather large one for an active alkaloid.   The difference between the accepted therapeutic dose and the toxic dose is fairly large, since as a rule the former does not exceed one grain daily for ten or twelve days, whereas the latter is probably not less than one grain a day for twenty-one days.   The more usual dosage of one-half to one grain of emetine per day for six or eight days is certainly on the safe side unless the patient shows an unusual susceptibility to the drug.   However, the onset of general lassitude and depression with intensified diarrhea, lessened reflexes and perhaps a neuritis are indications which appear in sufficient time to prevent continued treatment and serious results.   The very few fatalities among the many thousands of cases treated with emetine do not indicate it to be a dangerously toxic drug.   The real danger, as has been shown, lies in the too long continued use of therapeutic doses, an entirely unnecessary procedure in the treatment of amebiasis as the active endamebas will be destroyed by half

or one grain doses in six to twelve days, and the encysted forms, if present, will not be destroyed by continued emetine injections.

Since much has been written in an editorial way on the variation in the toxicity of emetine, and since most of the statements have been based on the article by Rowntree and Levy (2), it may not be out of place to comment on this subject. This paper by Rowntree and Levy was not on the toxicity of emetine, but on the variation in toxicity of commercial preparations, ampoules and tablets, of this alkaloid. Some of the editorials (9), however, misconstrued the statements of the article in question and dwelt at length on the variation in the toxicity of emetine and spoke of "impure emetine" and of "associated" or "dangerous impurities." As a matter of fact there are no associated impurities in ipecac which would increase the toxicity of emetine to anything like the extent indicated, for if all of the alkaloid should be cephaeline, the only other active constituent of ipecac, it would be only twice as toxic as pure emetine and would in no way account for the published results. It should be remarked in commenting on this article, that the fatality reported was not caused by the preparation of emetine claimed to be highly toxic, and that the patient, to whom this preparation was given, in very small dosage, did not exhibit symptoms which necessarily need be credited to the emetine. The most likely explanation of the authors' experimental results would seem to be more logically explained by a variation in susceptibility to emetine shown by dogs of various breeds and sizes, as were probably used in these experiments. An examination of table 1 in Rowntree and Levy's article will show that some of the dogs, receiving the so-called "unusually toxic preparation" A, required a total of 2.85 mgm. per kilogram to kill, whereas others required 11.7 mgm. per kilogram, a variation in toxicity of over 400 per cent when the same preparation was used. Still more contradictory is the claim for unusual toxicity of the preparation A, when the five commercial preparations were given to cats. If A is the same preparation found so toxic for dogs it is the least toxic of all when given to cats, requiring an average total of 30.25 mgm. per kilogram to kill. Furthermore, when given to cats, there

was less variation between the average toxic doses of the five commercial preparations (21.07 to 30.25 mgm. per kilogram) than there was in the variation among four cats receiving the same preparation, which in one instance (E) ranged from 14 to 27.24 mgm. per kilogram.

It need only be pointed out that emetine is a definite, relatively stable, alkaloid and that any increase in its toxicity can only be caused by an admixture of cephaeline which, even in large amounts, will not dangerously heighten the toxicity, or else by an abnormally low content of water of crystallization, which will cause only a slight increase in toxicity. Any real increase in toxicity of commercial emetine preparations can only be due to a mistake in compounding, which can be determined much better by a chemical analysis than by a biological test. ·

CONCLUSIONS

The substitution of the methyl group in emetine by radicals of the higher homologous alcohols markedly decreases the toxicity. Cephaeline iso-amyl ether is the least toxic of the series examined, being only one-fifth as toxic as emetine when given subcutaneously.

Emetine is not a very toxic alkaloid when given in a single dose, but is dangerous when given repeatedly in small doses over a considerable period of time.

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AGARICIN

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Commercial agaricin is obtained from powdered Polyporus Officinalis, a lichen which grows upon larch trees; further purification yields the active principle, pure agaric acid.

In the medicine of the middle ages the antihydrotic effect of the fungus (till recently known as Agaricus Albus) was known, and it was rediscovered by de Haen (1) in 1768. Young (2) in 1882 recommended commercial agaricin for use in the sweats of phthisis.

According to Jahns (3) the active principle of commercial agaricin is a resinous acid having the formula

$$C_{14}H_{27}(OH){<}^{COOH}_{COOH}.$$
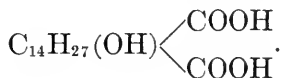
It is a crystalline body, very insoluble in cold water but the neutral alkali salts are easily soluble. The solution froths very readily and is irritating to mucous membranes and subcutaneous tissues. Hofmeister (4) made a careful pharmacological examination of the drug, using chiefly the sodium salt of pure agaric acid. He found that 25 to 50 mgm. injected into the lymph-sac of frogs caused general weakness, diminution of reflexes, stoppage of the respiration and cessation of skin secretion. The heart was first slowed, but this action did not begin till at least an hour after the injection; finally the heart stopped either in systole or diastole. Atropine did not antagonise the slowing nor did agaric acid antagonise muscarine. In the rabbit agaric acid excited the vagal center and slowed the heart. Large doses caused a rise of blood pressure. In the

cat after agaric acid, given subcutaneously or by the mouth, irritation of the peripheral sciatic stump did not cause sweating in the corresponding foot; nor was there any sweating when the cat after receiving agaric acid was placed in a heated chamber.

Hofmeister concluded that the drug affected the secretory apparatus itself and did not act centrally. He noted further that the salivary and tear secretions were rather excited than diminished by agaric acid and considered that it should not be united in one pharmacological group with atropine. He says that in man the action on the sweat glands only comes on some hours after administration but that it then remains effective for more than twenty-four hours.

Noguchi (5) reports that agaricin has marked haemolytic properties.

The efficacy of agaricin as an antihydrotic appears to be generally acknowledged but a satisfactory explanation of its action is up to the present wanting.

In the following notes the results of a further pharmacological examination of the drug are recorded, and a suggestion as to its modus operandi is made. The solutions used in the experiments were made with the sodium salt of pure agaric acid dissolved in frog's or ordinary Ringer. This solution is quite clear and froths very readily. All frogs were pithed.

Action upon the frog's heart perfused in situ from the sinus or the inferior cava, no fluid returning to the heart. The perfusing fluid contains 0.002 per cent of the sodium salt of agaric acid. With this dose there is at first an increase in systole, followed by rapid diminution of diastole and finally death in systole. The diastole also seems increased at first, but this is due to the throw of the lever. When the heart is perfused with a 0.001 per cent solution, there is a similar rise of systole and diminution of diastole, but the heart instead of dying in systole goes on beating with a much raised tone.

Action upon frog's heart of agaricin with adrenalin (fig. 2). It will be seen that the effect of agaricin in producing extreme systolic contraction of the frog's heart is not interfered with by perfusing with agaricin and adrenalin together. There are

however indications that adrenalin may increase the activity
of agaricin, for as shown in this figure, the systolic death is very
rapidly developed as compared with figure 1. Something of
the same kind has been observed with digitalis and adrenalin.
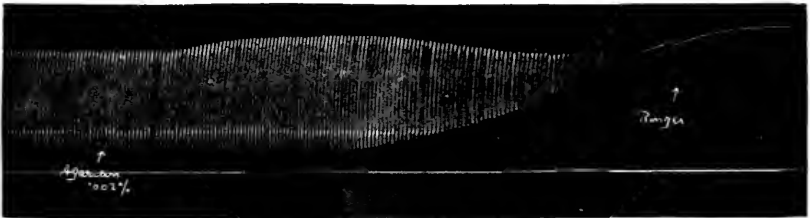
Fig. 1. Frog's Heart Perfused in Situ from Sinus

Upstroke systole. At first arrow, perfusion with 0.002 per cent sodium agarate
begun. At second arrow, Ringer substituted.
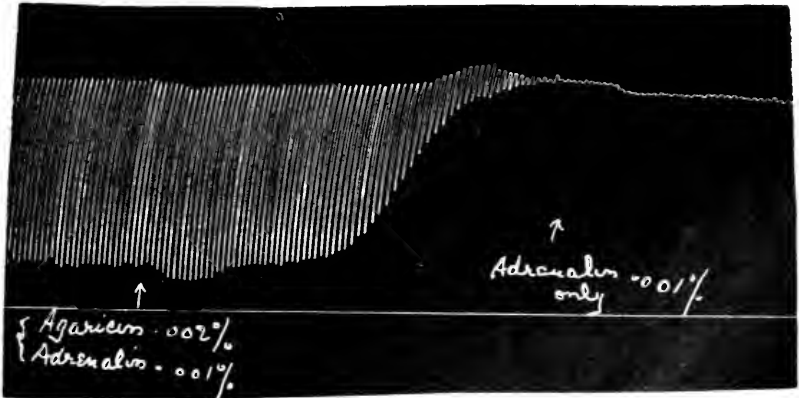
Fig. 2. Frog's Heart Perfused in Situ from Sinus
Upstroke systole.

Action of agaricin with chloral hydrate upon the frog's heart. As
is well known, chloral hydrate quickly stops the frog's heart
in diastole, and the action is generally considered to be upon
the muscle. There was therefore the possibility of an antago-
nism between chloral hydrate and agaricin. That such an an-

·tagonism in fact exists is strikingly shown in figure 3.   In *A*
the effect of perfusing a 0.3 per cent solution of chloral hydrate
through the heart is shown.   In *B* the same strength of chloral
hydrate is perfused with the addition of 0.005 per cent of agari-
cin.   The diastolic death of chloral is completely avoided and
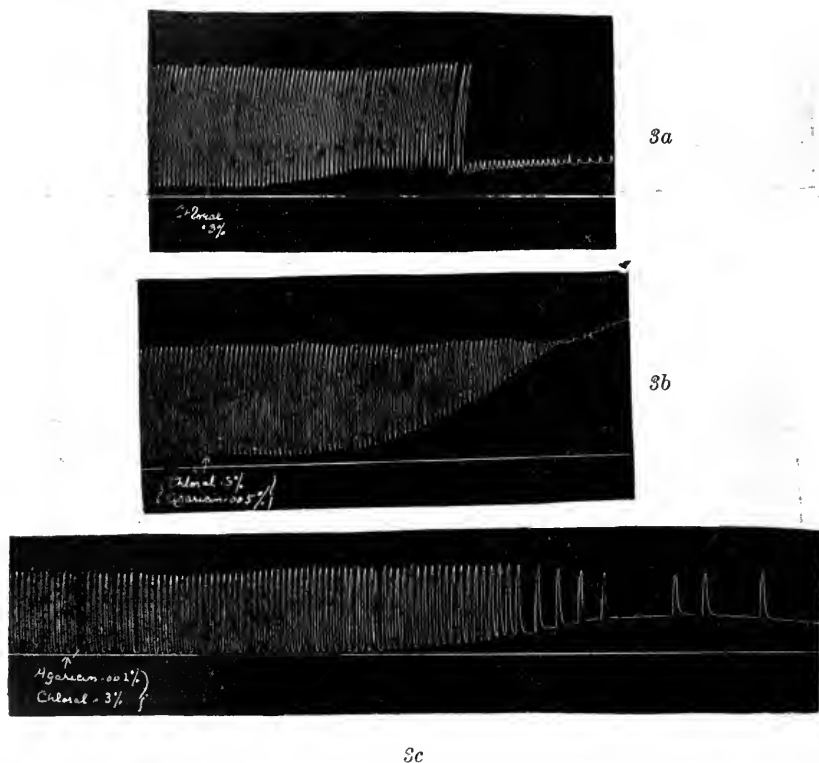
FIG. 3. FROG'S HEART PERFUSED IN SITU FROM SINUS

Upstroke systole.   Perfusion with Agaricin 0.002 per cent and Choral 0.3
per cent commenced at arrow.

the characteristic agaricin death in systole developed.   In *C*
the amount of agaricin is reduced to 0.002 per cent and now the
antagonism, though evident, is nothing like so complete. ·

Action of agaricin and pilocarpine upon the frog's heart.   There
is a certain degree of antagonism between agaricin and pilo-

carpine but this applies chiefly to the effect of the latter upon systole and much less if at all to its action in slowing the heart. Figure 4 shows the result of such a perfusion.
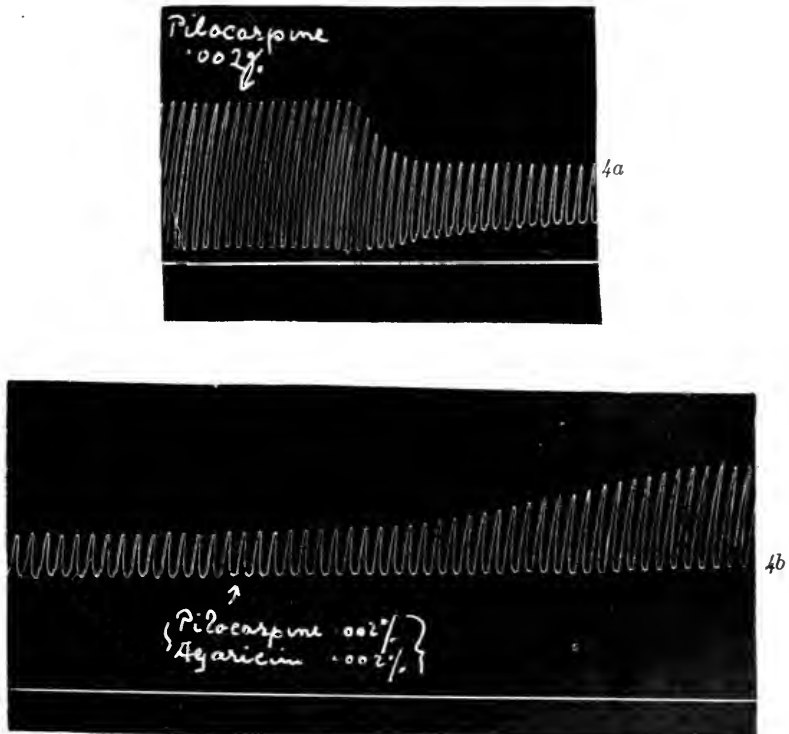
FIG. 4. FROG'S HEART PERFUSED IN SITU FROM SINUS

Upstroke systole. Interval of two minutes between *A* and *B*

Action upon frog's vessels; cannula in the aorta, perfusion through the limbs, outflow through the inferior cava, drops recorded. The results are shown in table 1.

TABLE 1

In three minutes before agaricin	33 drops =	11 drops per minute
In first three minutes after agaricin	21 drops =	7 drops per minute
In next three minutes after agaricin	6 drops =	2 drops per minute
In next three minutes after agaricin	1 drop =	$\frac{1}{3}$ drop per minute

The perfusing fluid contains 0.002 per cent of sodium agarate. There is marked diminution of the outflow, which finally almost ceases, indicating that the calibre of the vessels is greatly decreased. The muscle was, however, not dead, for the contraction gradually gave way under perfusion with Ringer alone though the restitution was remarkably slow.

Dixon (6) has shown that apocodeine first stimulates ganglion cells and finally paralyses sympathetic endings. In table 2 the effect of perfusing first with apocodeine till the arterioles relax is first given, then the result of changing the fluid to apocodeine with agaricin.

TABLE 2

In two minutes before apocodeine.............. 18 drops =	9 drops per minute
In two minutes after apocodeine.............. 12 drops =	6 drops per minute
After ten minutes perfusion with apocodeine,	
in two minutes............................ 34 drops =	17 drops per minute
In two minutes after apocodeine + agaricin... 6 drops =	3 drops per minute

The table demonstrates clearly that after perfusion with apocodeine and final paralysis of the sympathetic endings in the vessels, agaricin, added to the apocodeine solution, causes contraction of the vascular walls and diminution of the outflow. The constriction therefore appears due to direct action on the muscle of the arterioles.

Action upon a ring of frog's stomach. Figure 5 shows the effect of applying a 0.01 per cent solution of agaricin direct to a stomach ring. There is at first a marked rise of tone and some increase in peristaltic movements but it may be noted that, though not shown in the figure, a second application caused a further rise of tone which was, however, accompanied by a diminution in the size of the waves.

Action upon the excised intestine of a rabbit. A short length of the small intestine of a rabbit was suspended in warm oxygenated Ringer and without changing the solution a small quantity (1 cc.) of agaricin solution was added. The effects are shown in figure 6. It will be noticed that agaric acid 1:500,-000 caused considerable increase in peristalsis, whilst 1:40,000 markedly raised the tone and at the same time diminished the

range of movement. In another experiment in which the strength of agaricin was 1: 3,500, there was a very high rise of tone with complete cessation of the rhythmic movement.

In figure 7 the result of applying agaricin to the atropinised intestine of a rabbit is shown. The rise of tone takes place

FIG. 5. FROG'S STOMACH RING
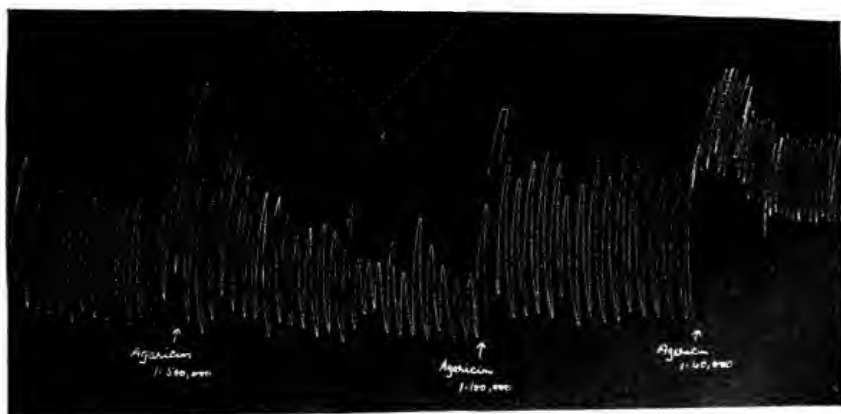
FIG. 6. RABBIT'S INTESTINE SUSPENDED IN WARM OXYGENATED RINGER

Upstroke contraction. At first arrow agaricin 1:500,000 added. At second arrow agaricin 1:100,000 added. At third arrow agaricin 1:40,000 added.

as usual and is very decidedly increased when the strength of the solution is raised to 1: 2,500.

Action of agaricin upon the excised uterus of the rabbit. When the excised uterus of a rabbit was suspended in warm oxygenated Ringer, the effect of the addition of agaricin was at first some

increase in movement, and little rise of tone. On making the strength of agaricin greater, there was more definite rise of tone,

FIG. 7. RABBIT'S INTESTINE

Upstroke contraction. Suspended in warm oxygenated Ringer. At first arrow, atropine added; at second arrow, agaricin 1:5,000 added; at third arrow, agaricin 1:2,500 added.
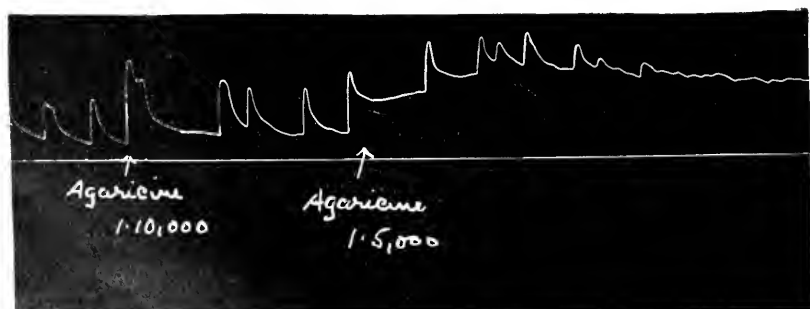
FIG. 8. RABBIT'S UTERUS (NON-PREGNANT)

and diminution of movement. Both these points are shown in figure 8.

Action upon the excised uterus of the cat. A portion of a non-pregnant uterus from a cat was suspended in warm oxygenated Ringer, and then adrenalin added. The result was the usual relaxation (fig. 9 a). Another portion of the same uterus under the same conditions was treated with agaric acid; there was marked rise of tone, but the movements were diminished rather than increased (fig. 9 b).
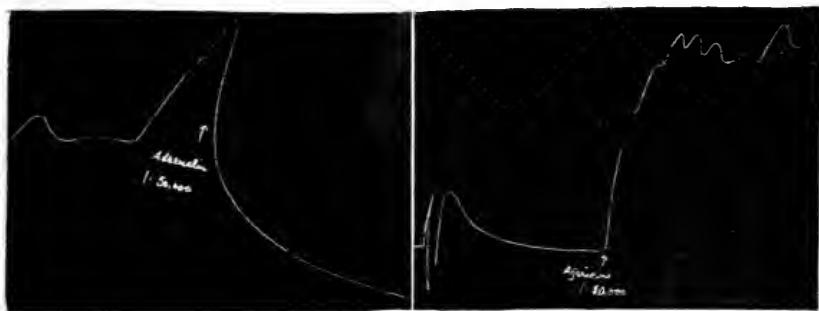
Fig. 9. Cat's Uterus (Non-Pregnant)
Both tracings are from the same uterus

Action of agaricin upon the excised bladders of cats and rabbits. Similar experiments were performed on the excised urinary bladders of cats and rabbits. In all cases the organs contracted on the addition of agaricin to the fluid in which they were suspended.

DISCUSSION

With reference to the heart, the above results appear to show clearly that agaricin is a heart muscle poison presenting some rather striking resemblances to the glucosides of the digitalis group and to saponin. It may be noted further that, like digitalis, once the action of agaricin upon the heart is fairly established it is extremely difficult to wash it away. With agaricin as with digitalis the auricles continue to beat vigorously after the ventricle has become so tightly contracted that little or no perfusion fluid can enter it. Such a ventricle contracted by agaricin is much less easily distended and restarted by rais-

ing the pressure than is the case with digitalis. All these points
tend to confirm the impression that agaricin is a powerful poison
for the heart muscle. As far as the heart nerves are concerned
there is no evidence that agaricin affects the sympathetic and
as to the vagus, under moderate doses of agaricin the diminu-
tion of diastole (rise of tone) is often accompanied by some
slowing which however is not affected by atropine and is there-
fore not due to action on the inhibitory apparatus in the heart.

There appears to be a certain amount of antagonism between
pilocarpine and agaricin. As has been frequently shown, pilo-
carpine, whilst slowing the frog's heart, also causes a consider-
able diminution in systole. Whatever this latter may be due
to, figure 4 shows that it is antagonised by agaricin.

Most striking of all is the very pronounced antagonism be-
tween agaricin and chloral hydrate in their effects upon the
heart. Chloral hydrate is acknowledged to act upon the heart
muscle and in view of the other facts it can hardly be doubted
that agaricin acts upon the same tissue but in an opposite way.
The contraction of the frog's vessels on perfusion with agaricin
solution might be due to stimulation of the augmentor sympa-
thetic nerve endings but, as shown in figure 4, after these nerves
have been paralysed by apocodeine, agaricin still constricts,
so that the action is almost certainly on the muscular tissue.

The effects of agaricin upon the frog's stomach and upon the
rabbit's intestine show that it does not stimulate sympathetic
endings (figs. 5, 6), and the fact that in the rabbit's intestine
its action is not cut out by atropine suggests that the increased
peristalsis and rise of tone are due to stimulation of the muscle
directly rather than an action like that of pilocarpine. In the
non-pregnant cat's uterus, which is relaxed by adrenalin, i.e.,
the inhibitor sympathetic is dominant, agaricin causes con-
traction, again indicating action upon muscular tissue, since
stimulation of the nervous apparatus would cause relaxation.
For the same reason, the action of agaricin upon the bladder
of cats and rabbits is almost certainly muscular and not nervous.

It appears then from the above that agaricin causes contrac-

tion and rise of tone in involuntary muscle irrespective of the nervous supply.

There remains to be considered what relation this muscular action of agaricin has to its power of preventing perspiration. Gaskell (7) says "the secretion of sweat is partly due to the contraction of a layer of unstriped muscle fibers which surround the sweat glands and by their contraction squeeze the sweat out of the gland." Although, as Barcroft and Piper (8) have shown, it is probably not possible to explain glandular secretion as entirely due to the squeezing action of contractile tissue, yet it seems not impossible that contraction and rise of tone of such tissue might obstruct or prevent excretion. As Hofmeister (l. c.) pointed out, the inhibitory action of agaricin comes on slowly, so that it has to be given some hours before its effect is required, but once established it remains for twenty-four hours or longer. This corresponds completely with the fact noted above that it is very difficult to wash away agaricin. The change which it produces in muscle is by no means an easily reversible one. The contraction and rise of tone of such a muscular layer would moreover tend to impede the free flow of blood which is generally necessary to increased functional activity. Further, if, as is probable, the muscular layer of the sweat glands carries out its squeezing function by means of rhythmic peristaltic movements these would be materially interfered with by a marked rise of tone.

SUMMARY

Agaricin is a powerful poison for involuntary muscle producing a marked and long continued rise of tone. It is suggested that this action affecting the muscular tissue surrounding the sweat glands prevents the secretion of the sweat, partly by more or less obstruction and by preventing peristaltic movements and partly perhaps by limiting the blood supply.

It is possible that the muscular tissue of the sweat glands may be especially susceptible to agaricin as the heart muscle is to the digitalis glucosides.

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ON THE TOXIC ACTION OF OPIUM ALKALOIDS INDIVIDUALLY AND IN COMBINATION WITH EACH OTHER ON PARAMECIA

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The protozoöcidal properties of emetin are well known and have established its value as a distinct therapeutic agent in various pathological conditions, more especially in amoebic dysentery. Recent work, however, by Vedder (1) and Rowntree and Levy (2) and others, has shown that that alkaloid is not devoid of toxic properties for the host, and serious poisoning has been reported following its administration. A search has therefore been made for other drugs which could take its place equally efficiently but with less danger to the patient.

Pick and Wasicky (3) noted the close chemical relationship between emetin and papaverin and in 1914 these authors published a comparative study of the toxicity of these two drugs for various protozoa, more especially the Paramecium, Colpidium, Ameba and Trypanosoma Brucei (Nagana). They found that papaverin was more toxic for Ameba than emetin, and was surpassed in that respect only by quinin. Inasmuch as the alkaloid papaverin and its salts have been shown to be less toxic and irritant than emetin the authors logically concluded that papaverin may be more useful than quinin or emetin in the treatment of amebic dysentery. The alkaloid narcotin was found by them to have properties very much the same as those of papaverin; a few experiments with morphin, however, showed that it had no effect upon most of the organisms studied.

In connection with an extensive and intensive study of opium alkaloids conducted by one of the present authors (M.) it was deemed interesting to investigate the protozoöcidal properties

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of all the opium alkaloids more closely.   Accordingly the present research was undertaken.

The purpose of this investigation was a threefold one.   In the first place a study was made of the toxicity or non-toxicity for protozoa of all the principal opium alkaloids and their derivatives.   In the second place, inasmuch as Straub (4), Macht (5) and others have pointed out that combinations of opium alkaloids produce synergistic effects unlike those of the individual components an inquiry was made into the effect of various combinations on the organisms studied.   In the third place an attempt was made to analyze more closely the mechanism of the toxic action of papaverin and the other alkaloids which were found to kill the microorganisms.

METHOD

For purposes of accurate comparison only one family of protozoa was studied, namely, Paramecidae (Bütschli).   Preliminary observations, moreover, soon indicated that the toxicity of various alkaloids differs with various species of paramecia. Further studies, therefore, were confined to a single species, namely Paramecium putrinum.   This organism is somewhat smaller and plumper than the common Paramecium caudatum, measuring 80 to 140 microns in length, and is somewhat more resistant than the latter (6).   The toxicity of any one alkaloid for this organism was found to be very constant and careful comparative studies could therefore be made with it.

The effects of the drugs were studied on hanging drop preparations.   For most purposes a dilution of the drug one part in one thousand was found to be the most convenient to employ. Occasionally, however, greater and lesser dilutions were used. Previous to the study of the drugs the normal movements of the organism were carefully studied and these having been once learned it was very easy to detect the earliest effects of poisoning.   These manifested themselves in changes either in the movements of the cilia and of the whole animal or in changes of form of the parasite or both.   The primary effect of the

poison was generally to render slower the ciliary movements or to paralyze them altogether so that the Paramecia moved about very sluggishly. Following this the shape of the animal tended gradually to become round, and with its death the protozoon finally disintegrated.

ACTION OF INDIVIDUAL ALKALOIDS

In respect to their effect on the Paramecium the opium alkaloids could be sharply divided into two classes—the morphin or pyridin-phenanthrene group and the papaverin or benzylisoquinoline group.

It was found that morphin has little effect upon the Paramecium. In no case was death of the organism noted sooner than seventy minutes after treatment with morphin sulphate or meconate and in most cases death was not observed at all within the period of observation of from one to two hours. Codein or methyl-morphin and thebain or dimethyl-morphin were never seen to kill the organism within the time studied '(two hours or more). Other derivatives of morphin, such as acetyl-morphin or heroin, ethyl-morphin or dionin, dihydrocodein or paracodein were found all to act in the same way. Apomorphin and apocodein have probably the same action, but owing to the rapid contamination of their solutions with chloromorphides and other oxidation products of morphin and codein their effect could not be conveniently studied. The only exception to the nontoxic action of morphin derivatives on paramecia was found to be benzyl-morphin or peronin. The explanation of this action will be considered later.

The action of papaverin and its closer relatives narcotin and narcein, on the other hand, was found to be diametrically opposite to that of morphin. Papaverin showed a greater toxicity for paramecia than even quinin, being surpassed only by emetin. Narcotin and narcein were also found to be quite toxic to the organisms. Tables 1 and 2 show the effects of the morphin and papaverin alkaloids on paramecia.

TABLE 1

	DILUTION	KILLED IN
		minutes
Papaverin hydrochloride................................	1 : 1000	23
Papaverin hydrochloride................................	1 : 10000	78
Papaverin hydrochloride................................	1 : 2000	46
Narcotin hydrochloride................................	1 : 1000	36
Narcotin hydrochloride................................	1 : 2000	52
Narcein hydrochloride................................	1 : 1000	37
Quinin sulphate................................	1 : 1000	34
Quinin sulphate................................	1 : 10000	79

TABLE 2

	DILUTION	ALIVE AFTER
		minutes
Morphin sulphate	1 : 1000	70
Codein phosphate................................	1 : 1000	90
Thebain hydrochloride................................	1 : 1000	90
Heroin hydrochloride................................	1 : 1000	90
Dionin hydrochloride................................	1 : 1000	90
Paracodein bitartrate................................	1 : 1000	90

ANALGESIC EFFECT

In connection with the action of papaverin and other members of the benzyl-isoquinoline group and of dionin belonging to the morphin group, a curious primary analgesic effect on the movements of the paramecia was noted. The organisms were seen to become anesthetized, their movements becoming sluggish and their general behavior suggesting a "narcotic" state. This peculiar condition was not identical with the toxic action of the various alkaloids inasmuch as it occurred, as stated, also after dionin which failed to kill the organism as late as two hours after its administration. It contrasted strikingly with the converse primary irritating effect noted after quinin and emetin. The effect of the drugs in this case seems to be a truly analgesic or anesthetic one and harmonizes well with observations of Macht, Johnson and Bollinger (7) on the effect of those alkaloids on the sensory nerve terminals.

EFFECT OF COMBINATIONS

In order to determine whether combinations of the opium alkaloids exhibited any synergistic effect upon paramecia a large number of combinations was studied. The following combinations were used: morphin plus papaverin, morphin plus narcotin, the proprietary preparation narcophin (morphin and narcotin meconates, one to two), codein plus papaverin, codein plus narcotin, papaverin plus narcotin, codein plus narcein, thebain plus papaverin, codein plus morphin, and pantopon or a combination of all the opium alkaloids. It was found that a

TABLE 3

	DILUTION	KILLED IN
		minutes
Morphin sulphate plus papaverin HCl (1:1)	1:1000	27
Morphin sulphate plus papaverin HCl (2:1)	1:1000	48
Morphin sulphate plus narcotin HCl (1:1) ..	1:1000	32
Narcophin...................................	1:1000	34
Codein phosphate plus papaverin HCl (1:1)	1:1000	25
Codein phosphate plus narcotin HCl (1:1)..	1:1000	26
Papaverin HCl plus narcotin HCl (1:1).....	1:1000	33
Codein phosphate plus narcein HCl (1:1)....	1:1000	17
Thebain HCl plus papaverin HCl (1:1)......	1:1000	30
Codein phosphate plus morphin sulphate (1:1)...................................	1:1000 Alive after $1\frac{1}{2}$ hours	
Pantopon...................................	1:1000 Killed in 31 minutes	

combination of two components belonging to different chemical series gave a synergistic effect; e.g., morphin plus narcotin or narcophin were more toxic than the arithmetical sum of the individual effects of their components. On the other hand, a combination of two alkaloids belonging to the same chemical series gave only an arithmetical combination and showed no potentiation or synergism. A combination of all the opium alkaloids such as pantopon produced a markedly synergistic effect. The action of the various combinations is illustrated by table 3. The effect of combinations here noted agrees well with Bürgi's theory concerning synergism (8).

ANALYSIS OF THE PAPAVERIN EFFECT

In a study of the action of opium alkaloids on the ureter published in this Journal, one of the authors (M.) showed that the papaverin effect on the ureter is due to the benzyl group contained in its molecule (9). The proof of this phenomenon was deduced from a comparative study of papaverin, narcotin, narcein, hydrastin and emetin on the one hand, and of cotarnin, hydrastinin and peronin or benzyl-morphin on the other. It was shown that papaverin and the other alkaloids which produced inhibition of the ureter all contain a double nucleus, namely a combination of iso-quinoline and benzyl groupings. On the other hand, those alkaloids in which the benzyl grouping was lacking and the isoquinoline grouping alone remained produced no inhibition and were even stimulating in their action. Further proof for the toxic action of the benzyl grouping was furnished by the behavior of peronin because while morphin alone does not inhibit the ureteral contractions but on the contrary stimulates them, peronin or benzyl morphin, on the other hand, produces paralysis.

In connection with the observations made on paramecia it was interesting to investigate whether the toxic action of papaverin was due to the benzyl constituent or not. With this end in view experiments were made on paramecia with hydrastin, hydrastinin, cotarnin and other substances. It was found that hydrastin and emetin produced exactly the same effect as papaverin, narcotin and narcein, that is, were very toxic to the organisms. On the other hand cotarnin hydrochloride (stypticin), cotarnin phthalate (styptol), and hydrastinin, alkaloids containing only the isoquinoline nucleus and lacking the benzyl grouping, were found to be entirely innocuous to the paramecia. These observations showed a striking analogy to the action of the same alkaloids on the ureter and pointed to the benzyl grouping as the toxophoric part of the papaverin molecule. Further corroboration of this theory was given by the action of peronin or benzyl morphin. It has already been pointed out that morphin and all its derivatives including morphin meconate were non-toxic to the paramecium. The poisonous action of

peronin is therefore obviously to be attributed to the presence of the benzyl grouping in that alkaloid.

As in the case of the ureter so in the case of the paramecia it was noted that the toxic action of the papaverin class of alkaloids was due to the *benzyl* grouping and not to an oxidized e.g., acidic aromatic radicle. Thus neither sodium benzoate nor sodium meconate, containing monacid aromatic radicles, nor cotarnin phthalate containing a diacid aromatic radicle produced any

TABLE 4

	DILUTION	REMARKS
Morphin sulphate...........................	1 : 1000	Alive after 70 minutes
Morphin meconate..........................	1 : 1000	Alive after 70 minutes
Methyl morphin (codein)...................	1 : 1000	Alive after 90 minutes
Dimethyl morphin (thebain)...............	1 : 1000	Alive after 90 minutes
Acetyl morphin (heroin)....................	1 : 1000	Alive after 70 minutes
Ethyl morphin (dionin)....................	1 : 1000	Alive after 70 minutes
Benzyl morphin (peronin).................	1 : 1000	Killed in 28 minutes
Narcotin HCl..............................	1 : 1000	Killed in 36 minutes
Cotarnin HCl (Stypticin).................	1 : 1000	Alive after 2 hours
Cotarnin phthalate (Styptol)..............	1 : 1000	Alive after $1\frac{1}{2}$ hours
Hydrastin HCl.............................	1 : 1000	Killed in 38 minutes
Hydrastinin HCl...........................	1 : 1000	Alive after $1\frac{1}{2}$ hours
Emetin HCl................................	1 : 1000	Killed in 19 minutes
Isoquinoline sulphate......................	1 : 1000	Alive after 1 hour
Benzyl acetate.............................	1 : 1000	Killed in 2 minutes
Benzyl benzoate (emulsion)................	1 : 1000	Killed in 5 minutes
Sodium benzoate..........................	1 : 1000	Alive after $1\frac{1}{2}$ hours

toxic action. The toxic effect was therefore due to the *unoxidized benzyl nucleus*. Through the kindness of Professor Reid of the Chemical Department of this University, the authors have been enabled to procure samples of benzyl acetate and benzyl benzoate and in that way to study the effect of the benzyl radicle still further. It was found that benzyl acetate in dilutions of 1 : 1000 killed the organisms in about two minutes, and even benzyl benzoate which is practically insoluble in water but of which an emulsion was prepared proved also toxic to the paramecia in a few minutes. On the other hand, the simple isoquinoline salt isoquinoline sulphate prepared by one of the authors was found to be non-toxic to the paramecia. It is thus evident that the

toxic effect of papaverin and its relatives is probably due to
its benzyl constituent.   Table 4 illustrates the effect of the va-
rious drugs used on analyzing the papaverin action and the sub-
joined formulae (figs. 1–7) render clearly the relationship in their
chemical structure.

$$CH_3O-C \quad CH \quad C \quad CH \quad CH$$

$$CH_3O-C \qquad CH$$

$$CH_3O-C \qquad N$$

$$CH \qquad C \qquad C$$

$$CH_2$$

Papaverin.

$$C$$

$$C \qquad CH$$

$$CH_3O-C \qquad CH$$

$$CH_3O-C$$

FIG. 1.

$$CH \qquad CH_2$$

$$C$$

$$O-C \qquad CH_2$$

$$H_2C$$

$$O-C \qquad N-CH_3$$

$$C$$

$$CH_3O-C \qquad CH$$

Narcotin.

$$O$$

$$\|$$

$$C-O$$

$$C \qquad CH$$

$$CH_3O-C$$

$$CH_3O-O \qquad C$$

$$CH \qquad CH$$

$$CH$$

FIG. 2.

$$C_{22}H_{23}NO_7 + H_2O + O = C_{10}H_{10}O_5 + C_{12}H_{15}NO_4$$

narcotin   opianic acid   cotarnin

FIG. 3.

Cotarnin.

FIG. 4.

Hydrastin.

FIG. 5.

$$C_{21}H_{21}NO_6 + H_2O + O = C_{10}H_{10}O_5 + C_{11}H_{13}NO_3$$

hydrastin   opianic acid   hydrastinin.

FIG. 6.

Hydrastinin.
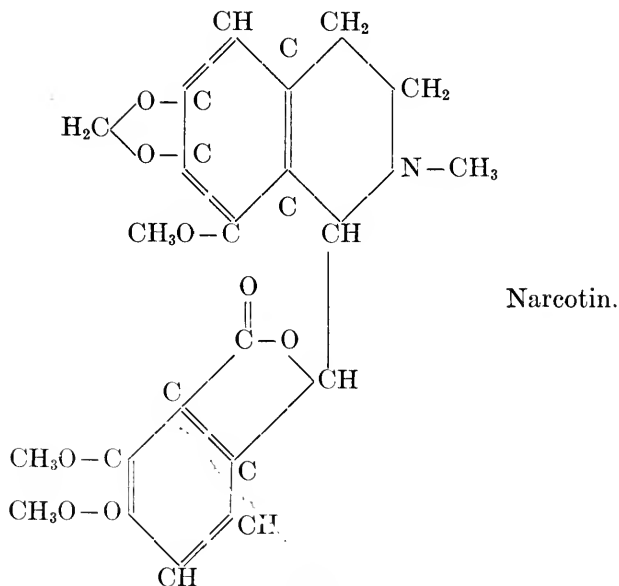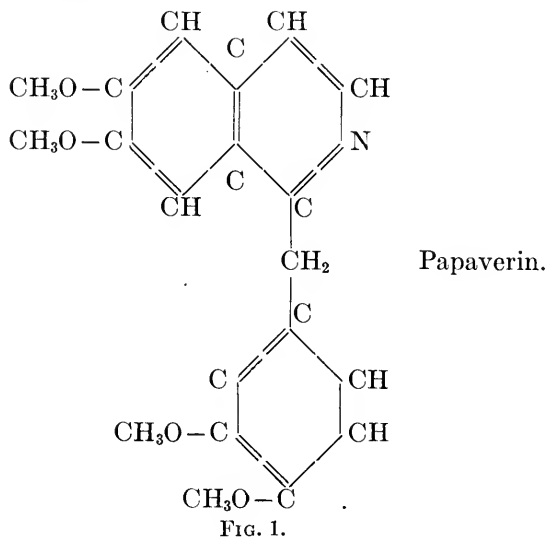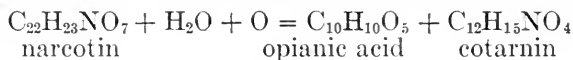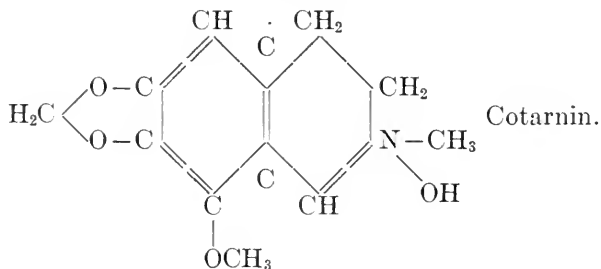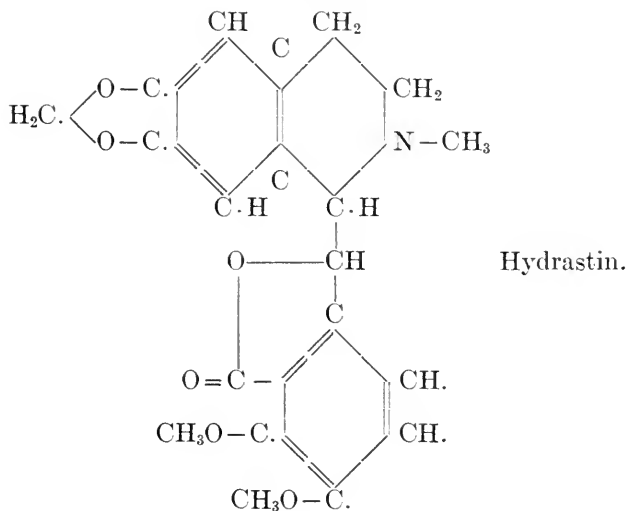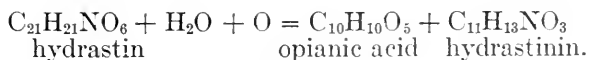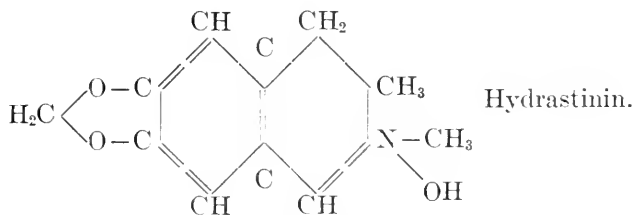
FIG. 7.

SUMMARY

The results of the above investigation may be summarized as follows:

1. The morphin group of opium alkaloids is non-toxic or very little toxic to Paramecium putrinum.

2. The papaverin group of opium alkaloids is very toxic to the Paramecium putrinum.

3. Combinations of members of the morphin and papaverin groups produce a synergistic effect in respect to their toxicity for the Paramecium.

4. Combinations of members of the same groups of alkaloids show no synergism.

5. The toxic action of papaverin and its related alkaloids for the Paramecium is due to the benzyl grouping, present in their molecules.

6. Papaverin and dionin and to a lesser degree narcotin and narcein show a narcotic or anesthetic effect on paramecia distinct from their toxic action.

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TOXICITY OF PHOSPHATES, IN RELATION TO BLOOD CALCIUM AND TETANY

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INTRODUCTION

The phosphate ions as they occur in solutions of orthophosphoric acid (H_3PO_4) and its various sodium salts (NaH_2PO_4; Na_2HPO_4; Na_3PO_4) have generally been regarded as devoid of pharmacologic action. Such changes as have been observed after the injection of phosphates were attributed to alkali or salt action or to the presence of impurities. But for the most part, previous investigators report negative results. In 1915 Greenwald (1) reviewed the more important literature on the pharmacology of orthophosphates and presented some data of his own from which he too draws entirely negative conclusions. He points out that Starkenstein (2) alone observed convulsions in rabbits after the administration of sodium phosphate—convulsions which could be prevented by the previous administration of calcium chloride. Greenwald emphasizes, however that Starkenstein (3) two years previously had published both facts and conclusions directly opposed to this and stated dogmatically that a specific toxic action of the phosphate kation cannot be demonstrated. The two experiments Starkenstein publishes in his first paper definitely show a toxic action of di- and tri-sodium phosphates on rabbits, similar to that of pyrophosphates. He concludes, that toxicity is not due to specific action of the kation, but rather to an alkali action. In his second paper he concludes, but does not prove, that toxicity is due to the precipitation of the calcium by phosphates as in the case of oxalates and fluorides.

The inorganic phosphates of the body are believed to exist chiefly in the form of sodium salts of orthophosphoric acid. Their physiologic importance in maintaining the neutrality of the blood and tissues owing to a remarkable capacity for binding acid and base, has been established in the classical studies of Sörensen (4) and of Henderson (5). More recently, variations of the inorganic phosphate content of the blood have been demonstrated in certain pathologic states. Marriott and Howland (6) found a phosphate retention in cases of nephritis with acidosis. Accompanying the high phosphate content of the blood, there is a marked diminution of serum calcium. Greenwald (7) discovered a phosphate retention in tetany following parathyroidectomy in dogs. The low blood calcium associated with this condition was first shown by McCallum and Voegtlin (8). Something in the nature of an antagonism between calcium and phosphate ions is suggested in these conditions.

A priori, it seemed, therefore, that introducing phosphates into the circulation might reduce the calcium content of the blood. And with a reduction of the circulating calcium, one might reasonably anticipate more or less profound physiologic changes.

METHODS.

Dogs were used throughout these experiments, save for one corroborative experiment on a rabbit. These animals were chosen, despite the fact that most of the previous pharmacologic studies of phosphates had been made on rabbits, because analytical work required drawing 10 to 20 cc. of blood at frequent intervals, and because dogs tolerate better than rabbits the injection of relatively large volumes of acid and alkali.

Blood was drawn with a pipette either by cannulating the femoral artery or by needle from the external jugular vein. The shed blood was allowed to clot in centrifuge tubes which were closed with rubber caps to prevent evaporation. Injection was made by cannulating for femoral or external saphenous veins. The cannula was attached to a large burette which

was connected above with a pressure pump. By adjusting two screw clamps, the rate of flow could be maintained at a constant and desired level. Cocaine (2 cc. of 0.25 per cent solution) was used subcutaneously as a local anesthetic when a vessel was cannulated. General anesthesia could not be employed for fear of inhibiting changes excited by the phosphate solutions. The dogs exhibited no evidence of pain with cocaine anesthesia. When a survival experiment was planned, the skin was sterilized with iodine and the wound flushed with hydrogen peroxide after removing cannulae. The incision was not sutured, as it was found to heal quickly and cleanly if allowed free access to the dog's tongue.

The phosphate solutions were prepared in two ways: (1) by titrating H_3PO_4 with NaOH to the required hydrogen ion concentration, (2) by mixtures (according to Sörensen's tables) of equimolecular solutions of $NaH_2PO_4\cdot4H_2O$ and $Na_2HPO_4\cdot2H_2O$. Where the latter method was used the reaction was subsequently checked by color comparison with standard phosphate mixtures, using the appropriate indicators. When differences occurred, the P_H determined by the indicator rather than the Sörensen table was taken as correct, because the reagents had not been recrystallized. The reason for using solutions prepared in two ways was to rule out the toxic action of impurities present in the reagents. The orthophosphoric acid used was tested for metaphosphoric acid with egg albumen. The test was negative. It was tested for arsenic and none was found.

No difference in the action of solutions from the two sources was observed except that those made from H_3PO_4 seemed more liable to induce vomiting than those prepared by mixtures of mono- and di-sodium phosphates. No explanation for this fact has been found.

The phosphorus content of all solutions was determined by titration with uranium nitrate, using potassium ferrocyanide as indicator. The figure thus obtained was regarded as the actual strength of the solution and all calculations of dosage were made on this basis. Solutions were warmed to body temperature (37.5°C.) before injection. After injection, blood

was drawn at fixed intervals and allowed to clot in centrifuge tubes. Clear serum was obtained by centrifugalization. Two cubic centimeters of serum were required for each calcium determination, one for phosphorus.

Calcium was determined by Marriott and Howland's (9) method; phosphorus by Marriott and Haessler's (10). The calcium method is somewhat difficult and painstaking. After some practice the method was found to work satisfactorily. In the writer's hands there was a maximal error of about 5 per cent; as this lies well within the limit of physiologic variation, the method seemed adequate. All determinations were done in duplicate. When the duplicates were not satisfactory, a third analysis was made. No difficulty was encountered with the phosphate method.

EXPERIMENTAL PART

To eliminate "salt action" and physiologic changes due to the injection of acid and alkaline solutions, phosphate mixtures were made up to the reaction of blood and approximately isotonic or hypotonic. Keeping the reaction constant, the dosage was varied in a series of experiments. All doses are calculated in milligrams of phosphorus per kilogram body weight. The condensed protocols follow:

Experiment. March 12. Dog 19. Fox terrier. ♂. Weight, 6.7 kgm. Solution made by titrating M H_3PO_4 with 0.1M NaOH. P_H 7.3. △ 0.248. Uranium titration = 1.59 mgm. phosphorus per cubic centimeter. Dog received 50 mgm. per kilogram.

2.03–3.04. Injection 210 cc. During injection there was slight restlessness and shivering.

3.00. Shivering more marked. Universal fairly coarse tremor and fibrillary twitchings in extensor muscles of upper part of fore leg. Dog unfastened, makes no effort to get up. Fore legs extended straight.

3.30. Condition has largely subsided. Slight tremor still present.

Analyses: milligrams calcium per 100 cc. serum:

Before injection,	10.2	⎤
One hour after injection,	7.8	⎬ Per cent of decrease, 23.
Three hours after injection,	8.0	⎦

In the following protocol the dose was again 50 mgm. phosphorus per kilogram, but the concentration of the solution was increased:

Experiment. March 15. Dog 20. Fox terrier. ♂. Weight, 4.8 kgm. Solution made by titrating 2M H_3PO_4 with 0.2M NaOH. P_H 7.4. △ 0.432. Uranium nitrate titration = 2.58 mgm. phosphorus per cubic centimeter. Dog received 50 mgm. per kilogram.

1.21–2.21. Injection 94 cc. After receiving 56 cc. dog exhibited marked shivering. No other symptoms.

Analyses: milligrams calcium per 100 cc. serum:

Before injection,	9.45 ⎫
One hour after injection,	7.70 ⎬ Per cent decrease, 19.
Three hours after injection,	8.00 ⎭

In the next experiment the dose was increased to 100 mgm. per kilogram. The same dog and same solution were used as in the first protocol cited.

Experiment. March 19. Dog 19. Fox terrier. ♂. Weight, 5.9 kgm. Solution made by titrating M H_3PO_4 with 0.1M NaOH. P_H 7.3. △ 0.248. Uranium nitrate titration = 1.59 mgm. phosphorus per cubic centimeter. Dog received 100 mgm. per kilogram.

2.05–3.00. Injection 374 cc. During injection dog shivered mildly.

3.22. Shivering more marked. Suddenly vomited watery material.

3.40. Few fibrillary twitchings in leg muscles.

4.30. Dog appears normal.

Analyses: milligrams calcium per 100 cc. serum:

Before injection,	9.45 ⎫
One hour after injection,	7.70 ⎬ Per cent decrease, 19.
Three hours after injection,	8.00 ⎭

In these three experiments a drop in serum calcium was observed, 19 per cent in two cases, 23 per cent in the third. Nothing very striking was noticed attending the calcium drop. One dog vomited. All shivered rather markedly. In two, fibrillary twitchings were seen. No cardio-respiratory changes were noticed. Fairly severe shivering is not an uncommon phenomenon in dogs which have been fastened to the laboratory table for any length of time. Such shivering is apt to be periodic, usually synchronous with inspiration. Shivering alone cannot be interpreted as a pathologic phenomenon.

The dose was still further increased with the hope of producing a greater calcium drop. Accordingly four experiments were done in each of which the dogs were given 150 mgm. phosphorus per kilogram, the phosphate mixture being made up to the reaction of the blood as before. The results were uniform and outspoken. A drop of from 41 to 51 per cent in serum calcium occurred; and a remarkable and striking succession of pathologic changes. These began as before with shivering and fibrillary muscular twitching. Vomiting followed and then a coarse tremor involving apparently all voluntary muscles including facial muscles, eye lids and tongue. The extremities, especially the forelegs, were stiffly extended, resembling a decerebrate rigidity difficult to reduce. Toes were widespread. Clonic spasmodic jerks occurred at frequent intervals. Pulse became accelerated. Respirations panting and temperature rose to 41°C. and over.

A typical condensed protocol of these four experiments is given below:

Experiment. March 22. Dog 20. Fox terrier. ♂. Weight, 4.5 kgm. Solution made by titrating 2M H_3PO_4 with 0.2M NaOH. P_H 7.4. Uranium nitrate titration = 2.58 mgm. phosphorus per cubic centimeter. Dog received 150 mgm. phosphorus per kilogram.

2.30–3.20. Injection 261 cc.
3.00. Shivering begins.
3.05. Fibrillary twitchings of muscles of legs.
3.13. Vomited.
4.20. Passed urine and feces.
4.30. Forelegs rigidly extended; coarse universal tremor.
4.35. Clonic jerks of extremities. Respiration panting. Temperature 41.5°C.
5.20. Condition the same.
5.50. Tremor has largely subsided. Dog lying quietly as if exhausted. Respirations deep and labored. Forelegs still rigidly extended

Analyses: milligrams calcium per 100 cc. serum:

Before injection,	11.3	
One hour after injection,	5.6	Per cent decrease, 51.
Two hours after injection,	5.9	
Three hours after injection,	5.9	

There can be no doubt of the toxic action of the solutions injected in these four experiments. The average calcium level before injection was 10.8 mgm. per 100 cc. serum; after, it was 6.1 mgm. or an average decrease of 44 per cent. Attending this, a syndrome of symptoms occurred closely analogous to the tetany following removal of parathyroid glands. Schäfer describes "tetania parathyreopriva" in these words:

> If the operation is complete, i.e., if it includes all four parathyroids, most animals die as the result of the removal; some within a few days, others within a few weeks. The most acute symptoms are exhibited by carnivora such as dogs, cats, foxes and wolves, and the young of herbivora, and are of a nervous nature. For the first day or two there are no symptoms other than some loss of appetite. There then supervenes marked exaltation of reflexes, which leads to the occurrence from time to time of fibrillar contractions of muscles and later cramp-like and clonic contractions and eventually convulsive fits; these may be of considerable violence and alternate with intervals of depression. The body temperature may rise 2 or 3°C. during the fit. The paroxysms are usually accompanied by rapid gasping respirations which may be synchronous with heart beats; sometimes by vomiting and diarrhoea.

The similarity of the two conditions is outspoken. It is obvious that we are dealing here with an acute experimental tetany. In this paper when the word "tetany" is used, it describes this pattern of physiologic changes. Shivering and occasional fibrillary muscular contractions are not regarded as conclusive evidence of tetany. With changes so striking and clean cut as occurred, it seemed unnecessary to employ methods of studying electrical reactions for evidence of the heightened neuromuscular irritability, characteristically appearing in tetany.

At this point we may conclude that orthophosphoric acid, when titrated with sodium hydroxide to the reaction of the blood and injected intravenously in quantities of 150 mgm. per kilogram calculated as phosphorus, at the rate of 12.75 mgm. phosphorus per minute (three experiments) or 62.8 mgm. phosphorus per minute (one experiment) will produce in dogs a condition of tetany.

To exclude the action of impurities, it seemed desirable to inject orthophosphoric acid and sodium hydrate separately. This was done. In neither case was tetany produced.

Control experiment. On April 3, Dog 19 received 400 cc. or 150 mgm. phosphorus per kilogram of the neutral phosphate (P_H 7.4). Tetany resulted. On April 12, the same dog received 430 cc. of orthophosphoric acid, again 150 mgm. phosphorus per kilogram and injected at the same rate. The P_H of this solution was 1.6. At no time was there tremor or rigidity. There was no gagging or vomiting. The temperature and respiration remained relatively constant, pulse rose from 102 to 124. At the end of the injection, the dog was lively and apparently well.

On May 2, the same dog was given intravenously an injection of the same sodium hydrate solution used April 3 to bring orthophosphoric acid up to the reaction of the blood; 447 cc. were injected. There was slight elevation of temperature, considerable hemolysis and hematuria. At no time were there signs of tetany.

It appears therefore that neither orthophosphoric acid nor sodium hydrate injected in the concentration and at the rate here employed will produce tetany. Any impurities which might be held responsible for the symptoms have been shown not to exist in the component reagents. The possibility that they are formed in the process of neutralization should be considered. If this were true one might think of pyro- and metaphosphates of sodium—both salts of known toxicity.

Control Experiment. May 15. Dog 27. Fox terrier. Weight, 6.550 kgm. Solution made from Kahlbaum's di-sodium phosphate—recrystallized three times from hot water. Tested for metaphosphates with egg albumen; negative. Dog received 150 mgm. phosphorus per kilogram of M/15 solution. He exhibited vomiting, diarrhoea, tremor and extensor rigidity.

This last control experiment we believe settles definitely the question of impurities. Any toxic action must be attributed to the specific chemical substance injected. It shows too that the di-sodium salt or secondary phosphate—in M/15 solution with P_H of 9.0—will excite an identical condition. Greenwald used the

tri-sodium or tertiary salt in $M/15$ concentration, as well as mixtures of mono- and di-sodium salts. It seemed desirable to repeat one of his experiments accurately. Accordingly a dog of approximately the same weight was chosen and received proportionate doses of $M/15$ Na_3PO_4 injected at the same rate. The results are in perfect accord with those of Greenwald with the exception that the dog vomited toward the end of injection. Signs of tetany were entirely wanting. The dog had received 77.2 mgm. phosphorus per kilogram; the blood calcium falling from 10.6 to 9.0 mgm. per 100 cc. serum.

It was concluded that the negative results were due to insufficient dosage. Eight days later the same dog was injected again with $M/15$ Na_3PO_4. This time he received 150 mgm. phosphorus per kilogram and was thrown into perfectly typical tetany. Serum calcium dropped from 10.1 mgm. to 6.1. Temperature, pulse and respiration before injection were:

        T 39.5°C.   P 88   R   18
One half hour later they were:
        T 42.5°C.   P 224   R 240

There was restlessness, struggling, vomiting, panting, salivation, clonic spasm, extensor rigidity and a curious stridulous cry suggesting a laryngeal spasm.

This experiment was repeated on another dog to rule out individual idiosyncracy. Results were entirely parallel. The animal received 442 cc. $M/15$ Na_3PO_4 or 161 mgm. phosphorus per kilogram. Tetany was violent. Temperature rose from 40°C. to 44.8°C. Respirations from 25 to 180 per minute. There was frothing at the mouth, extreme extensor rigidity, opisthotonos and death with immediate complete rigor. Calcium fell from 10.4 mgm. per 100 cc. serum to 5.5 mgm., and a concomitant rise in serum phosphorus was seen, from the normal level of 5.2 mgm. per 100 cc. serum to 16.

A brief review of the foregoing experiments leads us to the conclusion that the neutral (P_H 7.4) and alkali sodium salts of orthophosphoric acid intravenously injected are accompanied by a drop in serum calcium. The degree of drop depends upon

the amount of phosphates introduced. When the phosphates are injected in amounts equivalent to 150 mgm. phosphorus per kilogram the serum calcium drops from its normal fairly fixed level of 10 mgm. per 100 cc. to about 6 to 7 mgm. At this level (12) tetany is seen.

It seemed of interest and importance to investigate ortho-phosphoric acid and the acid phosphates as well. It has al-
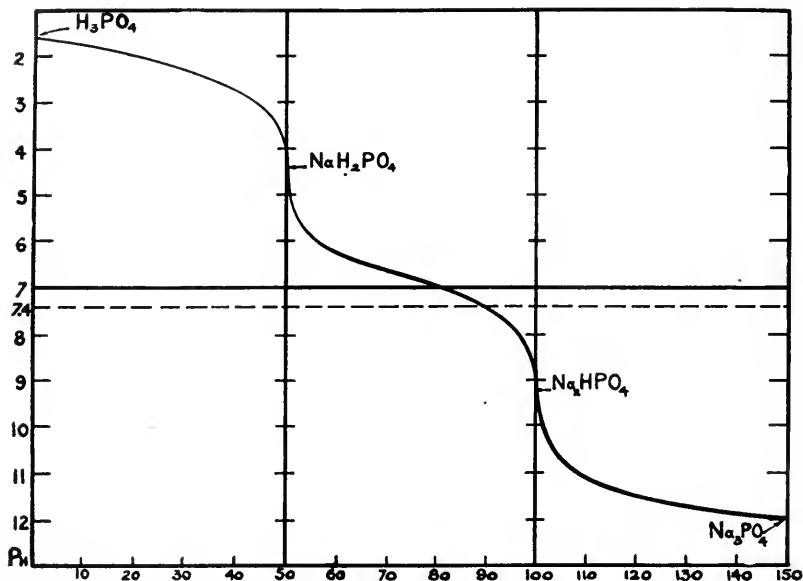
Fig 1. Titration Curve of Orthophosphoric Acid (After Clark and Lubs, Modified)

Ordinate represents concentration of H^+ ions; abscissa, cc. M/15 NaOH per 100 cc. M/10 H_3PO_4. P_H 7 = absolute neutrality. P_H 7.4 = reaction of blood. The region where sodium phosphate mixtures, in the concentrations and doses used in these experiments, will produce tetany, lies in the neighborhood of P_H 6.

ready been reported above that dog 19 received 150 mgm. phosphorus per kilogram in the form of orthophosphoric acid with no evidences of tetany. This was repeated on three other dogs. The results were consistently negative, save in one dog who died during injection. Death occurred unattended by symptoms of tetany. After the dog had received 440 cc. or 139

mgm. phosphorus per kilogram he suddenly grew cyanotic, gasped and died. In spite of the large volume of fluid infused, the animal exhibited a complete anuria, whereas a great diuresis was the rule in these injections. Autopsy showed a dilated heart, and much congested spleen and kidneys and we believe death may be attributed to cardio-vascular failure. It is perhaps of
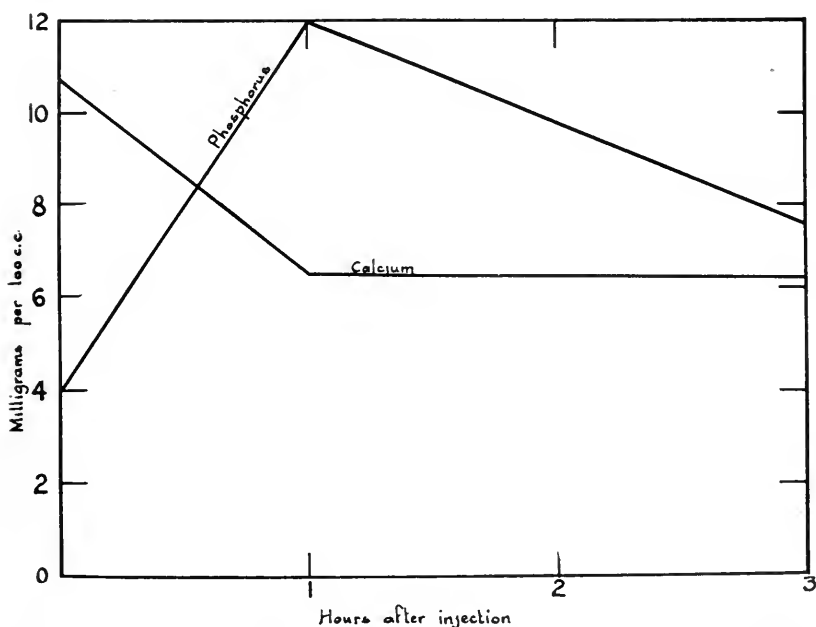
FIG. 2. CURVE REPRESENTS THE AVERAGE ANALYSES OF FOUR EXPERIMENTS DEMONSTRATING THE INVERSE CONCENTRATION OF SERUM CALCIUM AND PHOSPHORUS AFTER PHOSPHATE INJECTION

The ordinate represents milligrams per 100 cc. serum; abscissa, hours after injection. Analyses were made before injection and one and three hours after.

significance that the dog had a slightly elevated temperature, 40.5°C., before injection.

The acid phosphates were further investigated with like results. *It is to be particularly noticed that tetany did not appear despite the drop in serum calcium to the neighborhood of 6 mgm. per 100 cc.*

A series of experiments is presented in the following table (1) representing phosphate injections at various and decreasing H^+ ion concentrations. The point at which they become toxic and effect a condition of tetany apparently lies between P_H 5.6 and P_H 6.3, or in the neighborhood of P_H 6. A curve (fig. 1) (13) representing the titration of H_3PO_4 with NaOH at various H^+ ion concentrations is added to show graphically where this point lies in relation to absolute neutrality (P_H 7) and to blood alkalinity (P_H 7.4). A second curve (fig. 2) is appended—representing the average of four experiments—to show that an inverse proportion obtains between serum calcium and phosphorus after phosphate injection.

TABLE 1

Experiments to show that appearance of tetany depends upon reaction of phosphate solutions. With acid phosphates, tetany did not occur in spite of drop in serum calcium to "tetany level"

DATE 1917	DOG, SEX	WEIGHT	PHOSPHATE SOLUTION	REACTION P_H	MILLIGRAMS P PER KILOGRAM INJECTED	NUMBER CUBIC CENTIMETERS INJECTED	MILLIGRAMS Ca PER 100 CC. SERUM BEFORE INJECTION	MILLIGRAMS Ca AFTER	RESULT
		kgm.			mgm.	cc.	mgm.	mgm.	
April 30	27 ♂	6.3	H_3PO_4 M/15	1.6	250	772	10.8	6.6	No tetany
April 20	23 ♀	5.45	NaH_2PO_4 $4H_2O$ M/15	4.49	200	.	10.1	6.8	No tetany
April 26	25 ♂	5.75	NaH₂ 9.2 Na₂H 0.8 M/15	5.6	200	402	11.2	6.1	Slight tremor
May 1	28 ♂	5.95	NaH₂ 7 Na₂H 3 M/15	6.3	200	444	10.5	5.3	Tetany
March 27	20 ♀	7.50	H_3PO_4 + NaOH 0.1M	7.4	150	445	11.1	6.6	Tetany
April 23	24 ♂	6.3	Na_2HPO_4 M/15	8.8	200	631	12.5	5.5	Tetany
April 18	22 ♂	11.95	Na_3PO_4 M/15	12.4	150	793	10.1	6.1	Tetany

DISCUSSION

In the light of these experiments it is necessary briefly to reconsider the work of previous investigators.

Flack (14) was the first to inject phosphates into animals. His interest lay in the path of excretion. He reports three experiments on dogs, but draws no conclusions as to toxicity. Examination of the protocols shows that of the three dogs used, all gagged and vomited, one exhibited great weakness of the hind legs, and one cramp-like muscular contractions, salivation and general depression. He injected from 116 to 244 mgm. phosphorus per kilogram in the form of the tribasic salt. The results are not consistent, but the toxicity of the solutions cannot be questioned.

Gamgee, Priestly and Larmuth (15) were the first to study the pharmacology of phosphates. They had previously published the results of researches on the salts of vanadium and were struck with the differences of "poisonous intensity" in vanadic acid according as it occurs in ortho-, meta- and pyro-forms. The problem of phosphates they approached from the same point of view. They report marked toxic effect from meta- and pyrophosphates; none from the ortho-salts, which they describe as physiologically inert. They publish four experiments on intact animals, two on frogs, two on rabbits. The results of all were negative. One rabbit received 35.5 mgm. phosphorus per kilogram injected as Na_3PO_4. The weight of the second rabbit is not given so dosage cannot be calculated. It seems probable that negative results were due to insufficient dosage. In the one experiment in which we studied the effect of phosphates on the rabbit, 200 mgm. phosphorus per kilogram of $M/15$ Na_2HPO_4 excited typical tetany.

A year later Kobert (16) investigated orthophosphoric acid pharmacologically. Large doses, over 300 mgm. phosphorus per kilogram, he found could be injected with no untoward effects. Still larger doses caused death with symptoms of asphyxia as if from pulmonary emboli. Muscular contractions were observed at death. The di-sodium phosphate produced rise

in blood pressure and temperature and marked shivering. Kobert mentions the fact that morphine had been used. This may have inhibited more striking symptoms. He theorizes as to the mechanism of toxicity and believes that oxygen is bound by phosphoric acid and that the animals die of carbon dioxide poisoning.

Starkenstein's work has been referred to above. He observed restlessness, shivering and cramp-like contractions in rabbits. He attributed these to salt and alkali action and disclaimed any specific action for phosphate ions in the first paper. In the second, he groups phosphates with oxalates and fluorides and attributes toxic action to the common property of precipitating calcium.

The latest communication on the toxicity of orthophosphates is that of Greenwald. He asserts incorrectly that with the exception of Starkenstein's second paper, all previous investigators report negative results whereas we have found evidence of toxicity in the work of Flack, Kobert and in both of Starkenstein's communications. He adds experiments of his own which he regards as further evidence of the non-toxic properties of phosphates. These experiments have been considered in an earlier part of this paper.

The question of toxicity is a relative one. Even sodium chloride, if injected in sufficient quantities, is toxic. The manner of injection, dose, rate, concentration is of paramount importance. Negative statements as to toxicity must therefore be cautiously made and qualified with the exact experimental procedures employed.

The manner by which phosphates exert their toxic action is not clear. It is intimately bound up with the decrease in serum calcium, but this decrease, though obviously essential to the appearance of tetany, is not sufficient to produce it. Acid phosphates have been shown in this paper (see table 1) *to effect a like decrease without tetany.* This may bear some relation to the important researches of Wilson (17) and his co-workers who found that in tetany following removal of parathyroids a condition of "alkalosis" obtained and the tetanic attacks could be speedily relieved by acid injection.

We have not yet explained the mechanism of the calcium drop. Preliminary experiments on nephrectomized[1] and eviscerated animals suggest that calcium is not lost by excretion through the normal physiologic paths. This, together with interesting analogies drawn from experiments in vitro, points to an actual precipitation of calcium in the body, at least with the neutral and alkaline phosphates. It is conceivable that such precipitation, rather than calcium reduction per se, is causally related to the phenomenon of tetany—the conclusive experimental evidence for this hypothesis is wanting.

SUMMARY

1. Solutions of orthophosphoric acid and its sodium salts, when injected intravenously in dogs, cause a diminution in the amount of calcium in the serum.

2. The degree of diminution in calcium content or serum depends upon the amount of phosphate introduced.

3. When the phosphate solution is injected in amounts equivalent to 150 mgm. phosphorus per kilogram the serum calcium drops from its normal level of 10 mgm. per 100 cc. to approximately 6 mgm.

4. At this level a condition of tetany supervenes, provided the neutral or alkaline salts have been injected. With acid phosphate solutions, the calcium drop occurs unaccompanied by tetany.

5. The region at which the phosphate solutions become toxic when injected in the doses here employed lies in the neighborhood of P_H 6.0.

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[1] Nephrectomized dog injected with phosphate solution (P_H 7.4, 150 mgm. phosphorus per kilogram) showed calcium drop from 11.2 to 5.6 mgm. per 100 cc. serum, accompanied by typical tetany. Dog eviscerated under paraldehyde anesthesia—injected with phosphate solution (P_H 7.4, 150 mgm. phosphorus per kilogram). Calcium drop from 9.7 to 7.3 mgm. per 100 cc. serum. These two experiments indicate that a calcium drop can occur independent of the normal paths of excretion of calcium. It is obvious that before definite conclusions can be drawn from experiments of this nature, further work with phosphate solutions of different H^+ ion concentrations must be undertaken. Work along these lines is being continued.

CONCLUSIONS

The experimental observations recorded in this paper are believed to justify the following conclusions:

1. Orthophosphates of sodium, if injected in sufficient quantity, and at the right reaction, are definitely toxic substances producing in dogs a condition of tetany.

2. Toxicity is due to a specific action of the phosphate ions in combination with the reaction of the solution in which they exist.

3. The tetanic condition caused by phosphates is intimately associated with a decrease in serum calcium, but not dependent on this alone since a drop in calcium may occur without the appearance of tetany.

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PERFUSION OF THE MAMMALIAN MEDULLA: NOTE ON THE ACTION OF ETHYL ALCOHOL

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A review of the literature dealing with the action of ethyl alcohol in animal experimentation reveals the fact that very little attention has been paid to the response produced on respiration and particularly upon the respiratory center. It has accordingly seemed advisable to test the action of this substance on the respiratory center by the method of perfusing the mammalian medulla, which has previously been described (1).

In brief, this method consists in perfusing diluted defibrinated dog's blood through the common carotid artery of a small dog under such conditions of vessel ligature that the perfusate is practically isolated to the head region. The venous blood from the head is aerated and returned to the apparatus for repeated perfusion. The circulation in the trunk of the animal is preserved under artificial respiration, the nervous connections with the head alone being intact. Under these conditions alterations in the activity of the medullary centers produced by changes in the composition of the perfusate are recorded through the movements of the diaphragm and changes in the cardio-vascular mechanism. Such a procedure will maintain the vitality of the medullary centers for a period of two hours or more, which permits of experimental observations such as are described in this paper. It is obvious that the irritability of the nervous centers under observation cannot be assumed to be normal. It is probable that they are far below normal. Nevertheless they respond, when the experimental conditions are favorable, to reasonably small changes in the composition of the blood perfusate, as investigation has shown (2).

The results obtained indicate that alcohol has a definite effect upon the respiratory center causing increased respiratory activity. It is an open question whether this effect is to be ascribed to stimulation produced by the alcohol as such or to the production of an increased irritability so that the center responds more readily to the normal stimulus furnished by the circulating blood. The cardio-vascular centers exhibited a much less definite response; the arterial blood pressure was slightly elevated but no consistent variation in the pulse rate was observed.

In an exhaustive review of the literature up to 1900 Abel (3) found no convincing evidence that alcohol, however administered, causes increased respiratory activity by a direct action on the respiratory center. The increase in pulmonary ventilation incident to the administration of alcohol to human beings as well as to animals is to be ascribed not to a direct action on the respiratory center but to the increased metabolism and heat production brought about by the action of the substance on the tissues of the body generally. Abel likewise failed to find evidence that alcohol has an appreciable effect upon the heart-rate and arterial pressure. In connection with this report Abel performed numerous experiments on dogs in which he followed the respiration, heart-rate and arterial blood pressure, all of which confirmed his deductions from the earlier literature.

The conclusions reached by Abel in 1900 are in the main confirmed by the work done since that date and tend to support the hypothesis of Schmiedeberg that alcohol is essentially a depressant for all of the body functions. It is well known that Bintz (4) combats this point of view and contends that alcohol is primarily a stimulant for all nerve centers.

Dixon (5) in a more recent investigation lends support to the latter hypothesis. He reports that 0.1 per cent alcohol increases the force and rate of beat in the isolated rabbit's heart; that 10 cc. of 30 per cent alcohol administered intravenously to the decerebrate dog causes a slowing of the heart-rate which disappears on section of the vagi; that it causes a rise of arterial blood pressure accompanied by a decrease in volume of the limbs and abdominal organs. He paid no especial attention

to the respiratory effects, although one of his figures shows an
apparent increase in respiratory activity. His conclusion is,
however, that alcohol probably has a pronounced stimulating
effect upon all of the medullary centers.

Considerable interest is attached to the amount of alcohol
which may be recovered from the blood and urine of intoxi-
cated individuals inamuch as it indicates that the strength
of alcohol used in the present experiments does not exceed that
which may be found in clinical cases which recover from the
intoxication. Schweisheimer (6) reports finding as much as
0.2 per cent in the blood of comatose individuals and Widmark
(7) finds as much as 0.57 per cent in the urine. Widmark takes
the position that alcohol is not secreted by the kidneys but
passes from the blood to the urine by diffusion and that, there-
fore, the amount of alcohol found in the urine may be taken to
represent an amount previously present in the circulating
blood. He is of the opinion that symptoms of liquor intoxication
can scarcely appear until the concentration of alcohol in the
urine is over 0.1 per cent at least.

In an article which has recently appeared in this Journal,
Higgins presents a study of the action of ethyl alcohol on re-
spiration in man. Higgins used the alveolar carbon-dioxide
tension to indicate whether or not alcohol influences the irrita-
bility of the respiratory center. His experiments were care-
fully controlled and repeated a number of times on different
individuals with variations in the quantity of alcohol ingested.
He concludes that "alcohol sometimes acted to increase the
sensitivity of the respiratory center, as shown by a drop in the
alveolar carbon-dioxide tension; sometimes alcohol was without
action on the respiratory center."

The concentration of alcohol in the circulating blood when the
substance is taken by mouth must rise very slowly so that the
effects produced would be masked by a complicated change of
circumstances such that the results obtained would be difficult
of interpretation. The method employed in the present experi-
ments brings the alcohol in contact with the nerve cells very
promptly and accentuates the contrast between the control

and experimental periods. Furthermore the results obtained must represent the direct response of the centers since the chance of an indirect response through other tissues is at least limited to the head region and the records show a very prompt response when the change to the alcohol blood is made, the latency in evidence being due to the time required to pump the blood through the perfusion system.

Method. The control perfusate was prepared by diluting 180 cc. fresh defibrinated blood with 35 cc. Ringer's solution. The experimental perfusate was prepared in the same way except that known amounts of pure ethyl alcohol were first added to the Ringer's solution. The procedure consisted in perfusing the head with the control solution until conditions were constant when a change was made to the experimental solution. Temperature conditions were held constant by having both solutions in the same water bath. It is improbable that the small amounts of alcohol used appreciably altered the concentration or the reaction of the perfusate and tests to see if the alcohol caused any hemolysis of the blood were negative. The temperature, concentration, reaction and oxygen-capacity of the blood are, therefore, excluded·as causative factors in the results obtained, leaving only the alcohol which was added to the experimental blood to account for the stimulating action observed

Results. After several tests showing the stimulating effect of alcohol on the preparation incidental to other· work, two experiments were completed in which the action of alcohol was more particularly investigated. As the results obtained were essentially the same, it seemed unnecessary to extend the observations and the detailed consideration of one of these experiments will be adequate to substantiate the conclusions which are drawn from them.

Three records from this experiment are here reproduced. Figure 1 shows the response obtained on perfusing with 0.025 per cent alcohol. There is a definite increase in both rate and amplitude of respiration so that if the factor rate $\times$ amplitude be taken as a criterion of pulmonary ventilation, the increase of ventilation amounted to 110 per cent. Along with this increase

in respiratory activity there was a rise in both systolic and diastolic arterial blood pressures, the rise in diastolic pressure being slightly greater than the rise in systolic pressure. The heart-rate was not appreciably altered. Hence it is concluded that there was a definite increase in vasoconstrictor tone.

In the second test with alcohol 0.1 per cent (fig. 2), there was a still further stimulation of respiratory activity amounting to an increase of 278 per cent. Neither the blood pressure nor the heart-rate was affected.

In the third test with alcohol 0.2 per cent (fig. 3), there was a transitory increase of respiratory activity amounting to 30 per cent followed by a period of complete inhibition which lasted for more than ten minutes after perfusion with the alcoholic blood was stopped. The small excursions seen on the respiratory record in this period are due to the artificial respiration. In this test the record of arterial pressure is of no significance because a blood clot had formed in the recording system. It should perhaps be stated in this connection that the irritability of the preparation was undoubtedly below that which prevails under normal conditions of life. Under the circumstances we should expect that it would require a greater strength of alcohol to elicit an altered activity of the medullary centers. The response obtained with 0.025 per cent alcohol, therefore, does not signify that this strength of alcohol would be required to excite centers normal in irritability. On the contrary, it is probable that under normal conditions much less alcohol would produce an equally pronounced effect.

These experiments with ethyl alcohol fail to show any decided effect upon the heart-rate. The counts made from the records are as follows:

PER CENT ALCOHOL	RATE BEFORE	RATE DURING
0.025	104	112
	96	88
	172	172
0.05	60	60
0.1	184	184
	116	110
0.2	164	156

Fig. 1.

Ethyl alcohol 0.025%
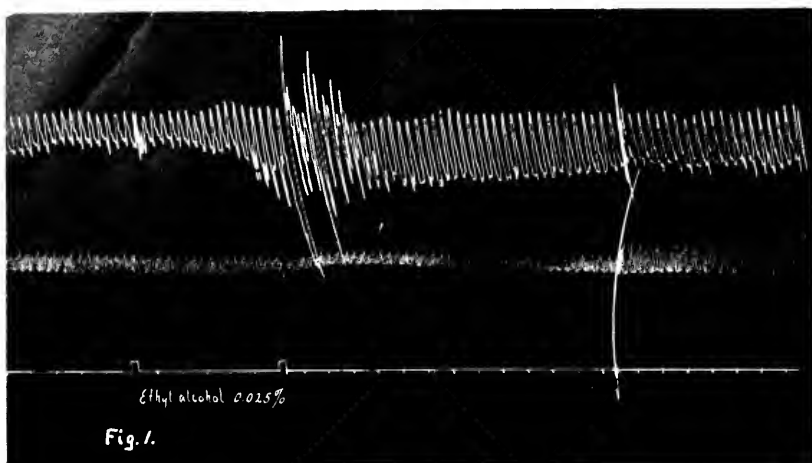
FIGS. 1, 2 AND 3. IN EACH FIGURE THE UPPER TRACING RECORDS THE RESPIRATORY MOVEMENTS OF THE EPIGASTRIUM

The middle tracing records the heart-rate and arterial blood pressure (Hürthle manometer). The lower tracing records time in five second periods; the upward movements of this signal delimit the period of perfusion with the alcohol blood.

Fig. 2.

Ethyl alcohol 0.1%

Fig. 3.
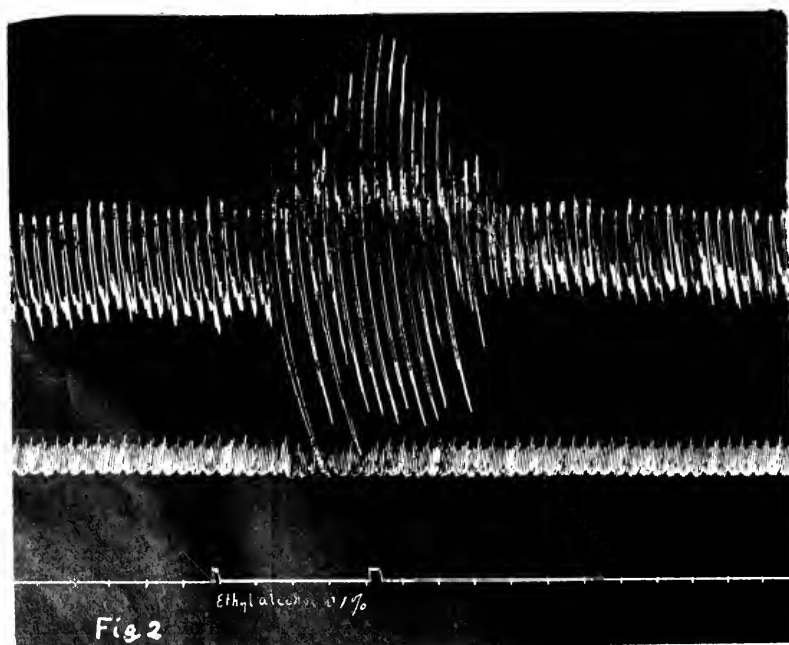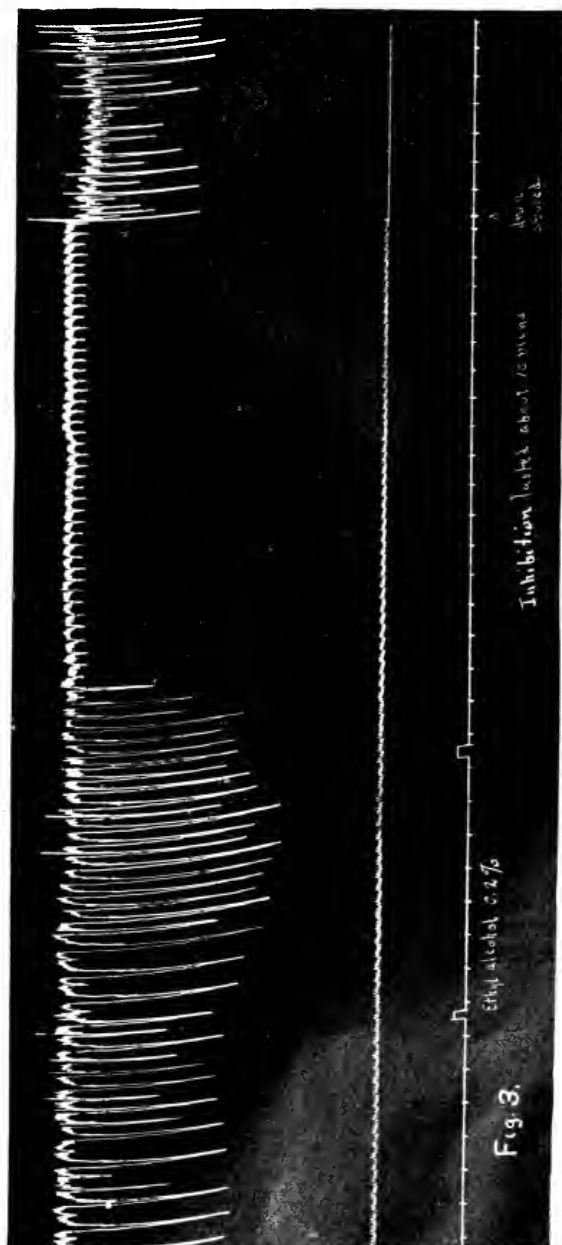
The rate per minute was increased once, decreased three times and unchanged three times. When a change in rate did occur, it was insignificant and might well have been caused by some other factor than the alcohol.

The arterial blood pressure was raised in two and unchanged in four observations. In both instances in which a rise of pressure occurred, the change was quite definite (see fig. 1) and indicates that this is probably the characteristic effect of alcohol on the vasomotor center.

SUMMARY

Perfusion of the medulla in the dog with defibrinated blood to which ethyl alcohol has been added to 0.025 per cent stimulates respiration. If the amount of alcohol be increased to 0.1 per cent, the respiratory response is greater than with 0.025 per cent alcohol. If the amount of alcohol be further increased to 0.2 per cent, a transitory stimulation occurs which is followed by a prolonged inhibition of respiration. Concomitant observations on the heart-rate and arterial blood pressure indicate that the former is not affected while the latter is raised.

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EXPERIMENTS WITH SUCCINATE AND ITS HYDROXY DERIVATIVES ON THE ISOLATED FROG HEART

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In a recent communication (1) embodying the results of studies on the isolated intestine it was shown that the introduction of the hydroxyl group into the succinate molecule modifies its physiological action. It was also stated that the effects differed in the large and in the small intestine and, as might be anticipated, varied with the concentration. The reaction of other organs to these compounds under similar conditions has never before been the subject of investigation although, as pointed out, studies have been conducted on the action of some members of this group, notably those of the isomers of sodium tartrate. Observations were therefore made on the isolated frog heart when perfused with these substances in order to fill a gap in the knowledge of the action of an important series of compounds and to gain some insight into the mechanism underlying the changes produced by them. This is especially desirable since the data thus far accumulated furnish no explanation of their behavior in the body, which is of special importance in this group of substances owing to their frequent occurrence in foods and various beverages, as well as on account of the use of some of them in medicine, or their occasional presence in the body under pathological conditions.

The method employed in these experiments was the same as that used in previous investigations on the frog heart which have been conducted in this laboratory (2).

Neutral salts free from impurities were used for the study of the action of the different compounds, due regard being had

to the effect of osmotic pressure which, as has been pointed out, causes depression. This was observed by Carlson (3) in experiments on the heart of limulus and was corroborated later by Salant and Livingston (4) on the frog heart. More recently this was also found to hold for the isolated intestine as reported by Salant, Mitchell and Schwartze (5). The salts were dissolved in Ringer's or Locke's solution and were either added to the other ingredients originally present or replaced equivalent amounts of NaCl in the solution, the resulting mixture in the one being hypertonic, in the other isotonic with frog's blood. It may be stated in advance that the changes produced by the hypertonic solutions were similar to those previously observed. In all cases freshly prepared solutions were employed for the perfusion tests. In case of the meso-tartrate and racemate perfusion was begun within six minutes to two hours after the salts were dissolved. This procedure, as will be stated more fully later, had to be resorted to as crystallization of the salts is likely to occur on standing.

EXPERIMENTS WITH SODIUM SUCCINATE

Perfusion of the heart with this salt was made for periods of half a minute to one hour with concentrations of N/100, N/50 and N/25 in Locke's or Ringer's solutions which were made up by replacing an equivalent amount of sodium chloride, or by adding it to the solution, the resulting mixture in this case being hypertonic. Except for a slight and transitory depression observed in rare cases when succinate was added to Locke's solution no effect could be noticed in experiments with low concentrations. The toxicity was appreciably augmented by increasing the amount of succinate in solution as marked depression was obtained with N/50 and N/25. This was due, however, to increased osmotic pressure, as the same concentrations of succinate when substituted for equi-molecular amounts of sodium chloride in Locke's solution produced a very moderate decrease of (fig. 1, A) the intensity of the contractions, the frequency not being affected. Recovery took place promptly when perfusion

with the salt was discontinued and was observed even after the heart had been exposed to the action of N/25 succinate for over an hour.

EXPERIMENTS WITH SODIUM MALATE

The tests were carried out as with the succinate, except that a wider range of concentration was employed. Solutions of N/200 and N/300 sodium malate caused depression which was progressively augmented as the amount of salt was increased. Thus N/100 malate perfused for a half minute caused a decrease of amplitude in one experiment amounting to 30 per cent. Although this effect was not as pronounced in other experiments it was nevertheless quite evident that this concentration of malate is depressing to the frog heart. In all of these experiments the force alone of the heart beat was decreased by malate. With solutions of N/50 and N/25 the amplitude was reduced 50 per cent in some experiments. This was especially the case with concentrations of N/25 (fig. 1, *B*) and occurred soon after the malate came in contact with the heart. After the maximum effect was reached no further change could be noticed, the same activity being maintained as long as the perfusion with the salt was continued, which was also observed in one experiment in which the perfusion with N/25 lasted sixty-two minutes. No after effects were noticed in any of the experiments, including those in which the concentration was quite high. Occasionally, however, the length of the perfusion time may cause more serious results. In one experiment that was perfused with N/25 malate for twenty-nine minutes heart action became weak, force, as well as frequency being decreased within one minute. The heart was irregular, groups of weak contractions being separated by long pauses. When perfusion with the malate was discontinued, the strength of the contractions increased, but they were in groups separated by periods of rest. It might be mentioned that the heart in this case was apparently normal before perfusion with malate was begun, the contractions being quite forcible and the rate uniform. The effect of hypertonicity was not very striking in these experiments but it was quite definite.
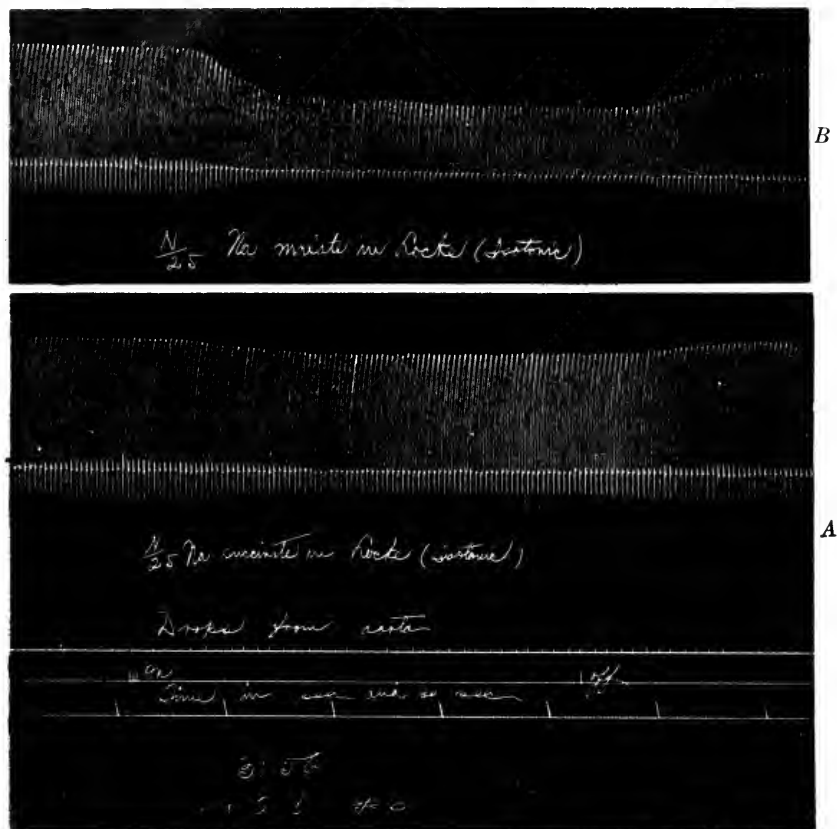
FIG. 1. FROG 218, SERIES I

Reduced one-fourth original size. The salts used in these experiments were dissolved in Locke's solution by replacing equivalent amounts of sodium chloride. The solutions are, therefore, slightly hypertonic. *A*, N/25 sodium succinate caused very moderate decrease of the intensity of the rhythm without changing its frequency. *B*, The same concentration of sodium malate shows much greater depression.

EXPERIMENTS WITH LEVO-TARTRATE

The behavior of the heart when subjected to the influence of levo-sodium tartrate indicated that it is much more active than any of the preceding salts, as depression of the rhythm frequently occurred when perfusion was made with low concentrations for periods of one half to two minutes, which was obtained with solutions in which the tartrate was added, as well as when it replaced an equivalent amount of sodium chloride of the Ringer's solution (fig. 2, A). It might be remarked at the outset that considerable variation in resistance was observed, some preparations having failed to respond to concentrations of tartrate which proved to be very active for other specimens. The first indication of depression was obtained with $N/300$ levo-tartrate which was isotonic with Ringer's solution. A slight decrease in the intensity of the contractions was the only effect produced in some of the experiments when the heart was perfused for two to four minutes. Recovery was observed as soon as perfusion with the salt was discontinued. Occasionally, however, even such a dilution produced complete inhibition. On the other hand we found that in some experiments the same treatment was without effect or even caused stimulation especially when the duration of the perfusion was not too short. The action of $N/200$ tartrate likewise showed that only a slight depression may be obtained in some experiments while the reaction in others may be very marked, the contractions being reduced in strength in one case 60 per cent, and entirely inhibited in another when perfused two minutes. In most instances, however, recovery was complete on discontinuing perfusion with the salt. The effect in general, which may be considered as the average result, was represented by a moderate increase of the intensity of the contractions and was distinctly greater than with $N/300$. With stronger solutions depression, though varying in degree, was always obtained and was in most cases very pronounced. Thus when $N/100$ levo-tartrate was perfused for two minutes complete inhibition of the ventricle occurred, while the contractions of the auricle became very feeble. Marked depression was also ob-
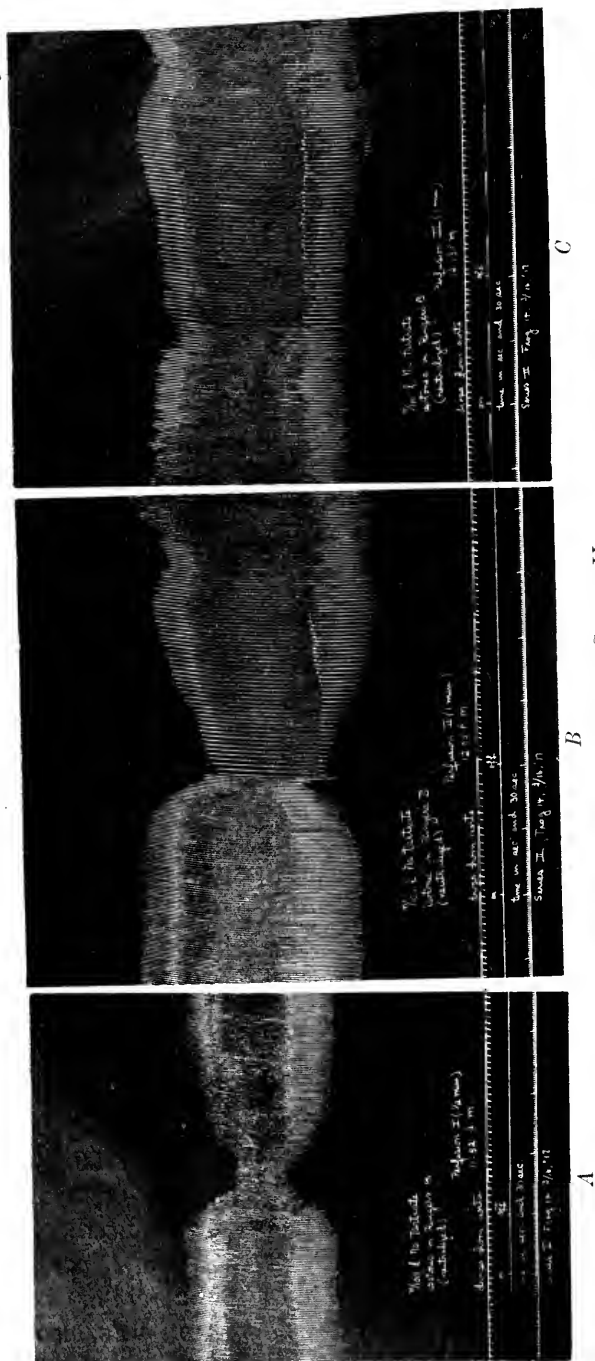
FIG. 2. FROG 14, SERIES II

Reduced approximately one-half of original size. Perfusion with levo-sodium tartrate in Ringer's solution. Salt substituted for equivalent amount of sodium chloride. *A*, Depression marked when the heart was perfused for the first time. *B*, The effect of a second perfusion less depressing. *C*, Third perfusion. Slight primary depression of amplitude soon followed by stimulation, but rate decreased during perfusion with tartrates and for some time after it was discontinued.

served when the heart was perfused for periods of one half to one minute (see figure 2, A). Complete arrest of the heart, which occurred within one minute, was produced when it was perfused with N/50 levo-tartrate. Recovery and sometimes stimulation was observed after perfusion with the salt was discontinued, the force, and sometimes also the frequency of the contractions being increased. In one experiment the amplitude showed an increase of almost 100 per cent. In all our experiments with this salt we had occasion to repeat the test a number of times in the same preparation. This brought out the very interesting fact that the reaction changed with successive perfusions which is shown very clearly in figure 2. It will be noticed that the first perfusion which lasted only half a minute produced a very marked decrease in the force of the contractions, the amplitude being reduced about 70 per cent. The depression produced by the second perfusion made thirteen minutes later was considerably less, while the third, carried out eight minutes after this, caused but a slight primary decrease of the intensity of the contraction and was followed by recovery and stimulation, the rate being reduced from 46 to 30 per minute which is a decrease of about 33 per cent. This effect was even better shown in experiments with weaker solutions. The very moderate depression which was sometimes observed after the first or second perfusion gradually disappeared as the treatment was repeated until the same operation finally caused the very opposite effect to that observed at the beginning of the experiment.

EXPERIMENTS WITH DEXTRO-TARTRATE

The effect of dextro-sodium tartrate on the isolated frog heart was much less than that produced by the sodium salt of levo-tartaric acid. An isotonic solution of N/100 in Ringer's solution caused a moderate degree of depression when perfused for two to eleven minutes (fig. 3). A primary decrease of the intensity of the contractions appeared almost as soon as the fluid entered the heart and continued in some experiments from one half to one minute, after which the heart steadily recovered although it

was still exposed to the action of tartrate. A stimulating after effect, moderate in extent, was also observed occasionally in this series of experiments.

The reaction to higher concentrations was studied with solutions of N/50 and N/25, both of which proved to be very active.
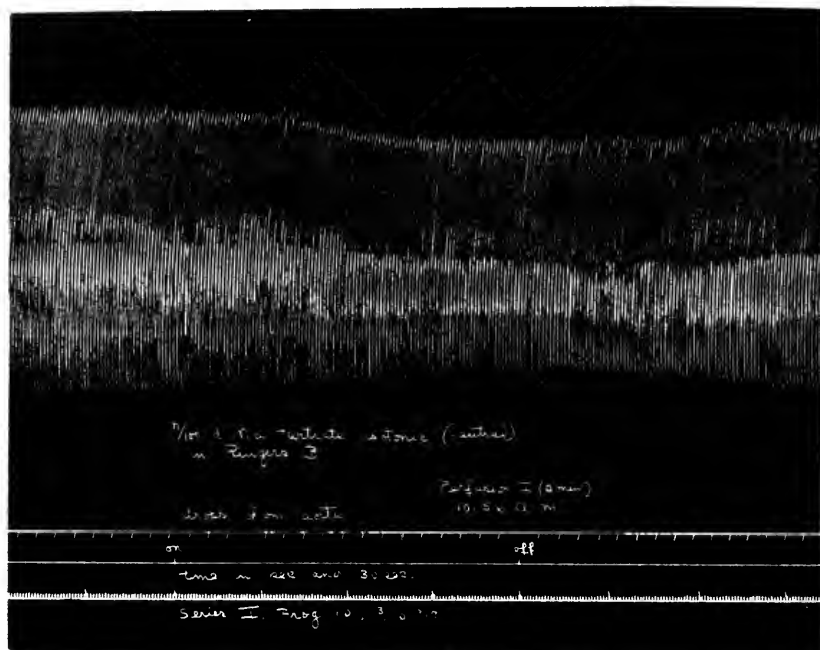
FIG. 3. FROG 10, SERIES II

Perfusion for two minutes with N/100 dextro-sodium tartrate which was substituted for an equivalent amount of sodium chloride in Ringer's solution. Depression by tartrate not marked. No after effect.

Perfusion for one minute with N/50 sodium tartrate caused a marked depression of the rhythm, involving rate, as well as the intensity of the beat. The effects obtained were more striking when the perfusion time was increased to four minutes. In one experiment with N/50 tartrate which was perfused for two minutes the initial depression was very pronounced, but this

lasted only about half a minute when steady improvement set in, the heart showing considerable stimulation at the end of the perfusion. The treatment with N/25 tartrate caused diastolic standstill in one experiment in which the perfusion lasted one minute. In another experiment in which the heart was perfused with the salt four minutes, marked depression, and at the end of three minutes, arrest of the heart in diastole resulted. In a third experiment the same treatment likewise caused pronounced cardiac depression, the amplitude being decreased nearly 70 per cent, but no arrest of the heart occurred. It may be observed that in this case six perfusions had been made previously and that treatment with the salt for a much shorter period earlier in the experiment produced complete arrest of the heart. Marked stimulation was observed when the perfusion with the tartrate was discontinued, and was much more pronounced than after N/100 tartrate. Hypertonic solutions, that is, tartrate that was added to Ringer's solution without replacing the sodium chloride caused greater depression. But it may be observed that this applies, however, to experiments with the higher concentrations. The depression after N/100 sodium tartrate was perfused was but slightly greater than that caused by the same concentration of tartrate when made up by replacing an equivalent amount of sodium chloride.

EXPERIMENTS WITH MESO-TARTRATE

When N/100 meso-tartrate in Ringer's solution, was perfused through a vigorous and normally contracting frog heart a reduction in the intensity of the contractions was the primary effect observed, which became evident soon after the solution came in contact with the heart (fig. 4). The depression increased for about thirty to forty seconds, then continued without change steadily for a period not exceeding half a minute and was followed by gradual improvement. This was best noticed when the perfusion time exceeded two minutes. The primary depression very frequently diminished with successive perfusions until the stimulating effect alone was noticeable. Thus in one experiment

a very slight depression was observed after perfusing the heart for one minute with N/100 meso-tartrate, but this was followed by a very pronounced stimulation. The most striking change which was uniformly observed in these experiments was the increased activity of the heart when the perfusion with the meso-tartrate
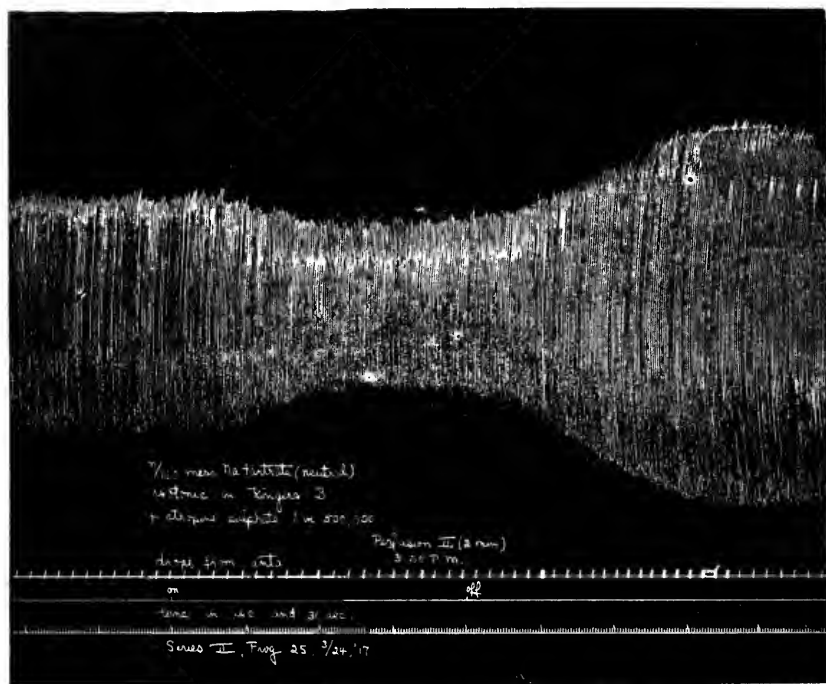
FIG. 4. FROG 25, SERIES II

Marked depression and a stimulating after effect when the heart was perfused with Ringer's solution containing N/200 sodium meso-tartrate for two minutes. The presence of 1 : 500,000 atropine sulphate in the solution failed to modify the action of the meso-tartrate.

was discontinued. The strength of the contractions was increased in some experiments more than twice that preceding perfusion with meso-tartrate. The frequency was also increased but this was not pronounced. Indeed, it was difficult to notice any difference in rate in some of the experiments. The

stimulating after effect lasted several minutes when the heart gradually recovered after reaching a maximum that persisted for about one minute.

Particularly interesting results were obtained with N/200 meso-tartrate. The primary depression observed with the stronger solution was slight or was entirely absent. Stimulation preceded by a long latent period when perfusion with the salt was discontinued was usually observed. Even hypertonic solutions, that is, those in which the meso-tartrate was added to Ringer's solution without changing its ingredients, indicated a stimulating effect as shown in the following experiment.

Frog 34, series III. N/200 meso-tartrate, hypertonic in Ringer's solution, was perfused for two minutes. Previously existing irregularity and heart block were abolished within one and one half minutes, and the heart action became much more frequent and the contractions stronger. No depression, but gradually increasing stimulation was observed almost immediately after perfusion with sodium meso-tartrate was begun. A second perfusion, carried out 8 minutes later with the same solution for a period of eight minutes produced the same effects, namely, increase in force and frequency of heart action. Irregularity also disappeared under the influence of meso-tartrate. After reaching a maximum, the heart action remained stationary for several minutes and then began to decline. A depressing after effect was observed in this case. It is important to observe that after a number of perfusions the heart may cease to react. The tolerance thus shown was even more clearly defined in these experiments as the stimulating effects likewise exhibited a considerable diminution.

EXPERIMENTS WITH RACEMATE

As the calcium salt was found to be very sparingly soluble, the test with sodium racemate presented considerable difficulty which made it necessary to confine our studies to experiments with low concentrations. A solution of N/100 was the highest concentration which could be used but even this was not

altogether satisfactory, as the appearance of crystals could be observed under the microscope at room temperature a half hour and sometimes ten minutes after it was dissolved in Ringer's solution, but no crystalline precipitate was formed in a solution of N/200 for several hours, and sometimes none could be found after twenty-four hours. The experiments were therefore carried out without unnecessary delay after the salt was dissolved, an interval of nine to twenty-eight minutes being allowed to elapse before the perfusion was begun. By determining the time of appearance of crystals under the microscope we were thus able to ascertain whether the changes observed were due to the salt or to the precipitation of calcium and thus guard against erroneous conclusions. For the sake of emphasis we wish to add that our results are based on the effects observed before precipitation occurred, the experiment being discontinued with the appearance of crystals under the microscope. The action of N/100 and N/200 sodium racemate (isotonic) consisted in depression of the intensity of the contractions which were more marked in experiments with the stronger solution. In both cases, especially when the period of perfusion exceeded two minutes a gradual improvement followed, while the heart was still under the influence of the salt. When a number of perfusions was made through the same heart a change in the reaction to racemate could be generally noticed. The depression observed at first was gradually decreased with successive perfusions. The effect of repetition was particularly interesting in experiments with N/200 racemate as the response of the heart observed under these conditions indicates that the salts may exert different effects in the same organ, at first depression, and later stimulation, preceded by a long, latent period.

DISCUSSION AND CONCLUSIONS

Before proceeding to attempt an analysis of the observations recorded in the preceding pages of this paper it may be of advantage to present a résumé of the experimental evidence brought forward to show the physiological relation and the chemical

constitution of the various salts. The facts established, there-
fore, may be briefly stated as follows:

The entrance of the hydroxyl group into the succinate molecule
produced marked changes in the action of this compound. The
depression, as already pointed out, was slight even when a con-
centration of N/25 succinate was perfused, but the effect was
noticeably greater with the same concentration of malate (see
fig. 1, A and B). By increasing the number of hydroxyl groups
a still greater depression was induced as N/100 levo-tartrate
(fig. 2) in Ringer's solution was found vastly more active than
a solution of malate of the same strength. The behavior of the
other isomers of tartrate shows that they are less active than the
levo, but the effect of each differed. The depression produced
by the racemate was less than that of the levo-tartrate but was
greater than that of the meso-tartrate, which may even cause
stimulation when a dilute solution is used. Dextro-tartrate
caused a very moderate degree of depression much less than
meso-tartrate after perfusion with N/100. The frequent oc-
currence of a marked stimulating after effect when perfusion with
tartrate was made may also be pointed out. Attention may be
called in this connection to the effect of repeated treatment with
the different isomers. It was frequently noticed that after
several tests were made on the same preparation the reaction to
the same salt became much weaker, the heart finally responding
by a very pronounced stimulation. Tolerance similar to that
obtained with tartrates, but no stimulation, was also reported
by Kuno (6) in experiments with different alcohols on the iso-
lated heart of the rabbit. He observed that the effect gradually
diminished with the number of perfusions and also with their
frequency. The depression caused by the first perfusion was
thus found to be greater than any of those which followed it.
Evidence of tolerance was also obtained when the perfusion
time was greatly prolonged, the marked primary depression in
this case having been followed by gradual improvement and in
some instances proceeded to recovery, which, it may be recalled,
was likewise found in the experiments with tartrates.

The reaction of the frog heart to the different salts may now be

considered in the light of the solubility of their calcium compounds. As exactly the same after effects were obtained with calcium free Ringer's solution and the isomers of sodium tartrate, it would appear that the disturbance of the calcium mechanism in the cell may be the cause of the changes produced by the salts of sodium tartrate, or is one of the factors concerned in the process. But this explanation would involve the inference that the toxicity of the salts would be parallel with the solubility of the calcium compounds. We had occasion to see this contradicted by the experimental evidence obtained in the present report since the calcium racemate was distinctly less soluble than the levo-tartrate which, as has been shown, was more depressing to the heart than any of the tartrates.

That the action of the so-called calcium precipitant on the heart is independent of the solubility of their calcium salts was also shown in experiments with oxalate, citrate and tartrate by Salant and Hecht (7). It would seem, therefore, that the calcium precipitation theory is likewise untenable in the present instance. Additional evidence against this view would also seem to be afforded by the tendency to tolerance and the stimulating effect which was noticed as a result of repeated perfusions with tartrates (see fig. 2). The depression of the isolated intestine of the rabbit produced by tartrates, as shown by Salant, Mitchell and Schwartze (8), likewise indicates that the effect of these salts cannot be attributed to the disturbance of the calcium mechanism for it has been found by McCallum (9) that calcium chloride inhibits intestinal peristalsis in intact mammals. This was also shown later by Starkenstein (10) in experiments on the isolated intestine. If precipitation of calcium occurred in the cell by the treatment with tartrates and other salts which form insoluble calcium compounds, stimulation would be the result, which was not the case. Although stimulation with weak and medium concentrations was induced in the small intestine of the rabbit, this, as was shown, was not the case when stronger solutions were used while in experiments on the large intestine depression always followed when any part of it was subjected to the influence of the salts in any concentration provided it was not very dilute.

No satisfactory explanation can be found at present of the striking similarity of the after effects produced when the heart is perfused with calcium free Ringer's solution and the tartrate or the other calcium precipitants such as citrate and oxalate. That different causes operate in each case is further made evident by the fact that repeated perfusions with calcium free Ringer's solution produced the same effect, no tolerance and stimulation being noticed when the heart was subjected a number of times to the influence of sodium and potassium ions only.

Considerable interest naturally attaches to the point of action of the different compounds, but the evidence at hand is inadequate for the formation of a satisfactory conclusion.   As was previously observed, however, in studies on the isolated intestine made with different organic salts there is reason to suppose that the mechanism is nervous in origin and not muscular.   It was pointed out that the different behavior of the large and of the small intestine when subjected to the influence of citrate, succinate and its derivatives, might be accounted for by assuming that the local inhibitory and motor sympathetic systems are stimulated simultaneously.   The action of these salts on the heart may likewise be due to the simultaneous stimulation of a motor and inhibitory mechanism.   In experiments with cholin on the isolated frog heart Golowinski (11) obtained an increase in the force of the contractions and a decrease in rate which he ascribed to the simultaneous stimulation of the vagus endings and Bidder's ganglia.   The stimulation caused by the salts under consideration was likewise restricted to the intensity of the rhythm, the rate being decreased at the same time.   It may be stated, however, that the vagus endings may be safely left out of account since perfusion of the isolated frog heart with atropine sulphate in Ringer's solution with or without tartrates failed to change the frequency of cardiac action (see fig. 4).   The seat of action must be sought, therefore, in a mechanism, the location of which is peripheral to the vagus endings.   According to Meltzer (12) inhibition is common to all irritable tissues and is as essential to life as stimulation.   The simultaneous stimulation of both of these mechanisms may produce the same effects as in the case

of the vagus and augmentors. It was shown by Bowditch (13), Heidenhain (14) and others that the inhibitory action appears first when the vagus and sympathetic are stimulated conjointly while the motor effect is manifested later. As regards the tolerance and stimulation which may be obtained with a previously depressing substance, it is possible that the effect here is similar to that observed by Gaskell (15) who maintained that vagus stimulation is not effective in the fatigued or in the poorly nourished heart.

The chief conclusions, together with the theoretical considerations contained in this report are briefly presented in the following résumé.

SUMMARY

1. Sodium succinate and its hydroxy derivatives are cardiac depressants, but weak solutions of some of the isomers of the salts of tartaric acid may produce stimulation.

2. The toxicity of the derivatives of succinate increases with the entrance of hydroxyl groups.

3. Stimulating after effects were observed in experiments with tartrates.

4. In experiments with repeated perfusions the depressing effects observed at first gradually diminished and stimulation was finally observed.

5. The evidence obtained in this investigation contradicts the calcium precipitation theory advanced in explanation of the mode of action of tartrates and other organic salts whose calcium compounds are soluble with difficulty.

The point of action of the salts is probably nervous in origin and not muscular.

7. Simultaneous stimulation of inhibitory and motor mechanisms is suggested to explain the different and opposite effects produced by some of the compounds.

8. The sodium salt of levo-tartaric acid was the most active of all the isomers of tartaric acid.

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ENDERMIC REACTIONS. III

FURTHER EXPERIMENTS ON LOCAL URTICARIA

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The first paper of this series described certain skin reactions produced by the local application of drugs. The second paper listed a series of organic chemicals that produce urticaria.

The present paper is concerned with certain further experiments on local urticarias, mainly with morphin and histamin.

I. DOES THE LOCAL URTICARIAL SENSITIZATION SPREAD TO A DISTANCE?

Török and Hári (1) found that glass capillaries filled with water alone produced an urticarial swelling when inserted into the skin of dogs in the neighborhood of morphin or atropin wheals. This appears as if the production of an urticaria sensitized the surrounding skin. However, these reactions do not appear to be perfectly constant, and are therefore not easy to interpret.

When applying water after mucuna to a normal skin, there is usually no reaction; or at most a very few pinhead papules. When the application was made in the neighborhood of a histamin or morphin wheal, the water often appeared to produce more frequent and larger papules than it does on normal skin. However, I have been able to convince myself that this is not due to an increased sensitiveness of the skin at a distance; but presumably to the accidental spreading of the histamin or morphin solution. At times there appears to be a sensitization; but when every precaution was taken, the response to water and mucuna was the same at a distance as in the neighborhood.

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In these experiments large wheals were produced by rubbing the skin with sand paper, carborundum stone or pumice stone, and then applying the 1 to 4 per cent morphin solution.

In one experiment, illustrating apparent sensitization, a large wheal was produced in this way, an inch below the elbow. Mucuna and water was applied above the elbow, between two and three inches from the morphin wheal. A similar application of mucuna and water was made to the corresponding area on the other arm. On the morphin arm numerous well marked papules of 2.5 mm. developed. On the other arm there were no papules.

In another experiment, it was aimed to exclude the spreading of the morphin solution entirely, by affixing strips of adhesive plaster an inch wide, and an inch apart on the fore-arm. Pumice and 1 per cent morphin were applied between the strips. After a good wheal had been obtained, mucuna and water were applied above and below the adhesive strips, about $2\frac{1}{2}$ inches away from the wheal, and to corresponding positions on the other arm. On both arms, the same reactions ensued. Towards the hand, 1.5 mm. papules; towards the elbow, 1 mm. papules.

In another experiment, the Hill method (see below) was used; dry blank punctures with a dry needle being made at various distances, above and below the morphin site, before and after the morphin punctures. The experiment is shown on the following table:

TABLE

DISTANCE FROM MIDDLE RADIAL CREASE		TIME OF PUNCTURE (P) TEN MINUTES BEFORE OBSERVATIONS (PAPULES OF MILLIMETERS) AT TEN MINUTE INTERVALS				
		12.55	1.05	1.15	1.25	1.35
cm.						
4.0	Blank	0.2–1	0.2–1	0–1	0–1	
6.5	Blank			P	0–1	0–1
8.5	Blank	0	0	0	0	0
10.0	Blank	P	1	0–1	0	0
12.0	Blank		P	1–2	0–1	0–1
15.0[1]	Morphin	P	6–8	7–9	7–9	7–9 (flatter)
17.0	Blank	P	1–2	1–2	0–1	0
19.0	Blank		P	1–2	0–1	0–1
21.0	Blank	1	1	1	0–1	
23.5	Blank			P	1	0–1
25.0	Elbow crease					
33.0	Blank	1	1	0		
35.0	Blank			P	1–2	0–1

[1] Four punctures made through 4 per cent solution of morphin.

The blank punctures raised wheals from 0–2 mm., quite irrespective of location (with reference to morphin) or time.

The experiment appears to furnish definite proof that there is no sensitization at a distance.

Hill's acupuncture Method.   Dr. H. W. Hill, of London, Canada, kindly suggested a method that he has used for vaccine and tuberculin inoculation (2).   The method was first used strictly according to Hill, putting a drop of the solution on the skin and making a slight puncture through this, by pushing the needle either flat or vertically against the skin.   The method was also modified by dipping the needle into the solution and puncturing the skin.   The results with the latter method were generally positive, although not always quite as good as when the puncture was made through the solution.   The solution is removed as soon as the puncture has been made.   The method causes a minimum of inconvenience of the subject, and gives a sharp, distinctive reaction.

The following results were obtained.

Morphin, 1 : 100 = 1–5 mm. papules.
Morphin, 1 : 500 = 1–2.5 mm. papules.
Histamin, 1 : 2,000 = 3 mm. papules.
Calcium chlorid, 10 per cent = 2.5–4 mm. papules.
Urea, 25 per cent (old solution) = 1–1.5 mm. papules.
Blank = 0 (less than $\frac{1}{4}$ mm. papules)
Atropin, 1 per cent = 1–2.5 mm. papules.
Formaldehyd, 4 per cent, = 3 mm. papules.
It is seen that all of the tests are positive.

II. LOCAL ANESTHESIA BY MORPHIN

It is generally believed by pharmacologists, that morphin does not possess a local anesthetic action.   The experiments of Macht (3) throw some doubt upon this.   I have found in confirmation of Macht that morphin wheals are distinctly less sensitive.   I was at first inclined to refer this to the edema. That, however, is not the explanation, for wheals produced by histamin are not anesthetic.

It does not necessarily follow from this that this local action has anything to do with the ordinary morphin analgesia. It requires fairly high concentrations of morphin, and is not complete even then. I have as yet formed no definite opinion on this point.

A typical experiment is the following: An area of forearm was rubbed with carborundum; and histamin, 1:1000, applied. A papilliform partly fused urticaria appeared The edema was not as marked as with morphin, 1:100.

On the other arm, an area was rubbed with carborundum stone and 1 per cent morphin applied. This produced a large, velvety, perfectly smooth wheal.

The sensitiveness of both wheals was tested roughly by a fine wire bristle. The touch of this on the normal skin was always perceived.

The histamin wheal distinguished the touch as accurately as the surrounding skin. With the morphin wheal, the touch was often not perceived. However, occasionally the touch was felt, but generally could not be accurately localized.

III. INFLUENCE OF DILUTION

The interpretation of the experiments on synergism and antagonism, which were planned, required some idea of the quantitative results to be obtained when the degree of action is varied by changing the concentration. The results showed that the degrees of action are not sharply delimited, and must, therefore, be interpreted with caution. Further complications are the variability of the skin at different times. However, large differences can usually be distinguished without doubt.

The experiments on the influence of dilution were made by the mucuna method, since this promises the most uniform scarification of the skin and is not usually followed by spontaneous edema. Hill's method would do as well.

The following experiments were made:

Morphin. 1:1000 produced 2 mm. papules, thickly enough to fuse. 1:2000, 4000, 8000; and water: these produced a few scattered 1 mm. papules; all practically alike, with all the solutions.

Histamin.   1: 10,000, 25,000, 50,000 and 100,000, all produced 2 mm. papules indistinguishable in the different solutions.

It is seen that with *Morphin* the action is very much decreased between 1: 1000 and 1: 2000. With *histamin*, the effects are practically identical with dilutions of 1: 10,000 and beyond.

IV. THE DETERIORATION OF HISTAMIN

The mucuna method sufficed to show the rapid deterioration of dilute histamin solutions.

A fresh solution of histamin of 1: 50,000 produced fair sized papules, after mucuna.   The solution was allowed to stand in a cork-stoppered bottle between 2 p.m. and 9.00 a.m., when it was again applied after mucuna.   This time it produced practically no reaction.

On the same day a freshly made histamin solution of the same strength applied after mucuna produced distinct papules of 2 mm. diameter.

V. SUMMATION OF ACTION

A series of experiments were made to determine whether the combination of two weak urticarigenic agents would produce any marked potentiation.   The results were negative.

The effects are practically like those of either agent used alone, meaning probably a simple addition.   The differences, if any, are within the limits of the delicacy of the method.

The experiments were made on the forearm after treatment with mucuna.   The solutions were applied together and in succession as follows:

Area (1). Solution of first drug.

Area (2). Solution of second drug.

Area (3). A mixture of equal parts of the solutions.

Area (4). The first drug; and when the effect was developed, the second drug.

Area (5). The second drug and when the action was developed, the first drug.

I. Morphin, 1: 1000—histamin, 1: 50,000.   All the areas are practically indistinguishable; 4 and 5 are perhaps slightly greater.   The effects are therefore at most simple addition.

II. Atropin, 1 per cent—formaldehyd, 4 per cent. The areas are nearly alike. However, the formaldehyd is rather more than the atropin; and the formaldehyd-atropin-mixture is intermediate. The successive application ((4) and (5)) is no greater.

Here again there is only simple addition of the effects.

III. Peptone, 1 per cent—urea, 25 per cent. The reactions are nearly alike. The successive application increases slightly. Again, the results are practically simple addition.

IV. Calcium chlorid, 10 per cent—formaldehyd, 4 per cent. All areas are practically alike.

V. *Heat to Morphin.* Application of a hot scalpel to the skin does not increase the reaction to morphin. (The heat was such that quite marked rubefaction remained over six hours.)

VI. ANTAGONISMS AND TREATMENT

The local effects of a few agents on urticarias were tried in the hope of throwing some light on the treatment and perhaps on the mechanism of urticarias:

Morphin-calcium chlorid. The first paper mentioned rather equivocal results. The following further experiments indicate that calcium neither increases nor diminishes the morphin effect:

a. Morphin, 1 per cent = 3 mm. papules. Then mucuna $+$ CaCl$_2$ = no change.

b. CaCl$_2$, 10 per cent = 1 mm. papules.

c. Morphin, 1 per cent in CaCl$_2$, 10 per cent = 2 mm. papules.

d. Morphin, 1 per cent = 3 mm. papules—then CaCl$_2$ = 10 per cent: apparently shrinks a trifle but remains larger than (*c*). Probably no change.

e. CaCl$_2$, 10 per cent = 1 mm. papules—then morphin, 1 per cent: The papules swell to 2 mm.

These negative results do not necessarily have any bearing on calcium treatment of urticaria, for in the latter the calcium is given systemically over long periods.

Morphin-barium. Barium was tried as a constrictor of smooth muscle. The previous work showed no results on normal skin by endermic methods. The present experiments also show no results on morphin urticaria.

 a. Morphin, 1 : 100 = 4 mm. papules.

 b. BaCl₂, 1 : 100 = 3 mm. papules.

 c. Morphin, 1 : 100 in BaCl₂, 1 : 100 = 3 mm. papules.

 d. Morphin, 1 : 100 = 3 mm. papules—then BaCl₂, 1 : 100 = no change.

 e. BaCl₂, 1 : 100 = 3 mm. papules—then morphin, 1 : 100 = no change.

 f. = as (*c*).

Influence of epinephrin on urticaria. This was discussed in the first paper. It was shown there, that epinephrin causes large urticarial wheals to pit and thus diminish in size. There was a question, however, as to the nature of this apparent antagonism. Further experiments have been made as follows:

1. Epinephrin applied to Urticarias

Experiment 6, mucuna method. Morphin, 1 : 100, produced 2 mm. papules. Applying to these epinephrin, 1 : 5,000, there was slight blanching but the papules were not noticeably diminished in size.

Experiment 7, mucuna method. Morphin, 1 : 1,000. and histamin, 1 : 50,000, were applied successively, producing papules. The area was then again rubbed with mucuna and epinephrin, 1 : 5,000. The papules blanch and flatten somewhat, but do not disappear.

Papules produced by atropin, 1 per cent, and others produced by formaldehyd, 4 per cent, after mucuna, are treated with mucuna and epinephrin, 1 : 5,000. The skin blanches somewhat, but the papules do not disappear any quicker than on the control.

Experiment 18. Urticaria is produced in one area by rubbing with carborundum, followed by mucuna and histamin; another area by mucuna and histamin. These areas, as well as a normal area are now rubbed with carborundum, and epinephrin (1 : 1000) applied. All the areas show marked blanching and goose-skin. The histamin swelling diminishes.

Experiment 19. A large, smooth wheal is produced by rubbing with carborundum and 1 per cent morphin. Carborundum is now rubbed at right angles through a portion of the morphin wheal, and into the normal skin, and epinephrin 1 : 1000 applied.

The whole epinephrin area blanches. On the normal skin, there is goose-flesh; on the morphin wheal, there is apparent pitting. There

is a definite decrease in the size of the morphin wheal, although it does not come down to normal.

Experiment 20. A large wheal is produced by rubbing a strip of skin with pumice stone followed by 1 per cent morphin. Through this at right angles and into the adjacent skin, another scarification is made with pumice stone and into this is rubbed epinephrin, 1:1000. The morphin wheal begins to pit and then blanches. In doing this, it quite evidently contracts. The final disappearance of the morphin, however, is not faster than with that portion of the wheal that had not been treated with epinephrin.

2. Morphin applied after epinephrin

Experiment 6–E. Mucuna and epinephrin, 1:5,000, produced blanching. When morphin, 1:100, is now applied to this area, it produces 2 mm. papules, the same as on the untreated skin.

Experiment 21–B. A strip of skin is rubbed with pumice stone and epinephrin, 1:1000. This results in marked blanching and goose-skin. It is noted that a mottled blanching occurs outside of the goose-skin area, without any pitting; indicating that there is a direct vascular action which apparently occurs with solutions more diluted than are needed to contract the skin muscles.

Across a portion of the epinephrin area, mucuna and morphin (1:100) were applied. This produced a quite good confluent wheal, which is quite as good on the epinephrin as on the surrounding skin.

It was noted that the goose-flesh disappears from the epinephrin around the margin of the morphin area.

3. Morphin and epinephrin together

Experiment 6–C and F. Mucuna was applied followed by morphin, 1:100, and epinephrin, 1:5,000. This produced 2 to 3 mm. papules, just the same as with morphin alone.

Experiment 19. Two scarifications were made to form a cross. To one, epinephrin, 1:1,000, was applied; to the other, morphin, 1:100, so that both drugs were applied at the intersection. No morphin wheal was produced anywhere; but at the area of intersection, the epinephrin paling and pitting was distinctly smaller.

Experiment 20. This was arranged similar to the preceding, employing pumice stone in place of the carborundum. In this case, the morphin produced some, although not very marked edema, which was quite as great on the adjacent skin as that over the epinephrin.

It may be concluded that morphin and epinephrin are somewhat antagonistic, but that the effects of either may occur in the presence of the other. It would seem that the vascular action of epinephrin and of morphin are antagonistic; and that the morphin may therefore completely remove the epinephrin effect. On the other hand, it does not seem as if the epinephrin acts directly on the morphin edema, and therefore it cannot antagonize the action of the morphin completely. It flattens the wheals but does this apparently by muscular contraction. The epinephrin blanching persists longer than the morphin edema whether the two have been applied on each other or separately.

This speaks against the assumption of Jadassohn and Rothe (Berl. klin. Woch., 1914, p. 519), that epinephrin prevents urticaria by arresting the arterial blood supply whilst leaving the lymphatic outflow unimpaired.

VII. REPEATED APPLICATIONS AND TOLERANCE

As discussed in the first paper, urticaria is conceived by most investigators as a dermatitis, differing from other dermatites, by its limited severity and duration. Some experiments of Jadassohn and Rothe suggest that this might be due to a rapidly acquired immunity of the lesion to the irritant. If this were true, repeated applications to the same area should produce less and less effect; if the fading of an urticaria is due to its immunity towards the irritant, then it should of course fail to react towards application of the irritant.

This was tested by several methods, with the result that the reaction appeared to become smaller in some cases. This, however, was shown to be due merely to diminished absorption in the edematous area; for when effective scarification was produced, the fading wheals reacted quite as strongly, if not more strongly, than the normal skin.

The experiments were as follows:

Experiment 15. An area was rubbed fairly heavily with sand paper and then with 4 per cent morphin, producing a large wheal over the entire area. A number of additional applications of the morphin were made to one-half of this area at intervals of five to ten minutes, but without scarification. This produced no further effect.

Experiment 16. A heavy application of mucuna was made and 1 per cent morphin rubbed on, producing 2 mm. discrete papules. Five further applications of morphin were made to the same area at ten minute intervals; the alternating ones with mucuna. Notwithstanding this, the urticaria gradually disappeared; simulating an immunity.

Now a half of this morphinized area and an adjacent portion of the skin that had not been treated with morphin were lightly sand-papered, and 4 per cent morphin applied. This produced a good urticarial reaction which was even greater on the previously treated skin than on the untreated skin—perhaps because the slight swelling in the treated gave a better contact with the sand paper.

Experiment 18. A large histamin wheal was produced by rubbing with carborundum stone and histamin, 1:1000, at 2:15. At 2:50, the eruption had begun to fade a trifle. Mucuna and histamin were now applied to a part of this wheal and also to the normal skin. The previous scarified layer reacted much more. The greater reaction is doubtless due to the better penetration of the mucuna through the abraded skin. However, it is evident that the skin has not become immune.

Experiment of April 25, 1917. A histamin wheal was produced by Hill's method. This wheal was reinoculated by the same method at intervals: the wheal became much larger, considerably higher and lasted much longer. There was evidently no decrease of sensitivity.

It appears from these experiments that the limited reaction of urticaria is not due to acquired immunity. Evidently, the agents themselves are not capable of producing the *severer* phenomena of inflammation; at least in concentrations that can come into play.

VIII. URTICARIGENIC AGENTS ON EXCISED HUMAN SKIN

Freshly excised skin from a human breast was stretched on cork, stabbed in places with a scalpel, and the following solutions applied to the separate punctures:

Morphin... 1:100
Calcium chlorid...................................... 10 per cent
Urea.. 25 per cent
Peptone... 1 per cent
Formaldehyd... 4 per cent

None produced any visible effect.

CONCLUSIONS

1. Local urticarigenic agents do not sensitize the skin at a distance toward water.

2. The local application of morphin to the scarified skin produces a distinct though limited analgesia.

3. The severity of urticarial reaction does not decrease in strict proportion to the dilution.

4. However, the difference of the urticarial reaction is sufficient to show the rapid deterioration of dilute histamin solutions.

5. The combination of several weak urticarigenic agents does not produce marked potentiation.

6. The morphin urticaria is not materially influenced by the local application of calcium or barium chlorid.

Epinephrin and morphin are somewhat antagonistic in their local vascular actions. Morphin may therefore remove the epinephrin blanching; but epinephrin cannot remove the morphin edema effectively.

7. The fading of a drug urticaria is not due to rapidly acquired tolerance. Repeated applications do not increase the severity of the urticaria beyond a certain point; but this seems to be due to inherent limitations in the drug-irritation.

8. Excised human skin is not altered by the usual urticarigenic agents.

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THE EFFECT OF CAFFEINE ON THE REACTION TO CARBON DIOXIDE OF THE NORMAL HUMAN RESPIRATORY MECHANISM[1]

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INTRODUCTION

In previous papers Edsall and Means (1) and Higgins and Means (2), considered the effects of several drugs, including caffeine, on the gaseous exchange and pulmonary ventilation of normal human subjects. Higgins and Means also studied the alveolar carbon dioxide tension under the same circumstances. A fall, definite though rather slight, was nearly always found after a subject had received caffeine. This was interpreted to mean that caffeine rendered the respiratory center more sensitive, for it seemed unlikely that an acidosis could be produced in a short time as result of giving a small dose of such a drug.

It was with the hope of throwing further light on the subject that the present research was undertaken. We have here employed the reaction to increasing amounts of carbon dioxide in the inspired air, as a criterion for judging the sensitivity of the respiratory center.

It was our plan originally to make an extensive study along these lines and to include a number of the commonly used drugs. We were obliged, however, because of the pressure of other work,

to discontinue our experiments after the completion of the work on caffeine, which we are consequently reporting at the present time.

The previous work on the effect of caffeine on the respiration differs markedly from the experimental work on the effects of this drug on other bodily systems. Notably in the case of the circulation are discrepancies and disagreements, not only as to conclusions, but also as to experimental results, the rule. On the respiration, however, caffeine has always been thought to have a stimulating action. Binz (3) reported an increased respiratory rate in animals after the administration of caffeine and Cushney (4) confirmed this finding, noting that the average depth is greater. The latter noted in his experiments the variability in the action of this drug which seems to be one of its chief characteristics. He also found that the reaction to CO_2 was changed under the influence of the drug and deduced that this change was due to the rapidity of the anabolism in the center, the catabolic discharge remaining approximately constant. Heinz (5) found an increase in the depth of respiration after caffeine as well as an increase in rate but his method, involving the use of valves, is such as to vitiate his results.

A rise in the total gas exchange after caffeine has been noted by various investigators and the literature on the subject has been reviewed in a paper by Means, Aub, and DuBois (6).

METHODS OF EXPERIMENTATION AND TABULATION

The subjects of this investigation were both normal men, physicians.

G. P. G. is twenty-four years old, is 163 cm. in height and has an average weight of 55 kgm.

J. H. M. is thirty-one years old, 175 cm. in height and has an average weight of 75 kgm.

The experiments were all done with the subjects lying flat on the back, at complete muscular rest, but no attempt was made to have them in the nüchtern condition.

The caffeine was taken by mouth in the form of the pure alkaloid dissolved in water. The dosage given in the accompanying table therefore is in terms of the pure alkaloid.

The apparatus used was that of Benedict (7), the carbon dioxide absorber being replaced by a galvanized iron box of about fifty liters capacity, which thus formed a large dead space so that the carbon dioxide instead of being removed, slowly accumulated. Samples of air were withdrawn at frequent intervals from the box and analyzed for carbon dioxide in the Haldane apparatus. A quantitative graphic record of the breathing was obtained from the spirometer.

The method of tabulating the results is one originated by Peabody (8) and subsequently used by Newburgh, Means and Porter (9) and by Means and Balboni (10). The ventilation of the lungs while the subject was breathing room air was called 100 per cent. The higher ventilation when the subject was breathing carbon dioxide is expressed as a percentage of that when he was breathing room air. The percentage is called the ventilation coefficient (represented by V in the text figures). When the ventilation is doubled the coefficient is 200, etc.

The ventilation is calculated from the kymograph record at the percentages of CO_2 existing in the box when the samples were drawn. The points thus obtained were all plotted on cross section paper, the ordinates representing percentages of CO_2 and the abscissae the ventilation coefficient. In order that the figures might be compared, averaged, etc., the most probable curve was drawn through the points obtained and the ventilation coefficients at the even percentages of CO_2 read off. The ventilation coefficients given in both tables and in text figure 1 were obtained in this manner. In text figure 2 the points as originally determined are plotted.

Usually a single reaction was obtained on one day, either a normal control, or following the taking of caffeine. In experiments 15, 16 and 17, however, reactions were obtained both before and after taking the drug, on a single day.

DISCUSSION OF RESULTS

The data of the various experiments are collected in table 1. A study of the reactions to carbon dioxide will show that there is certainly no definite tendency for the curve to rise more steeply after the subject has taken caffeine. This is demon-
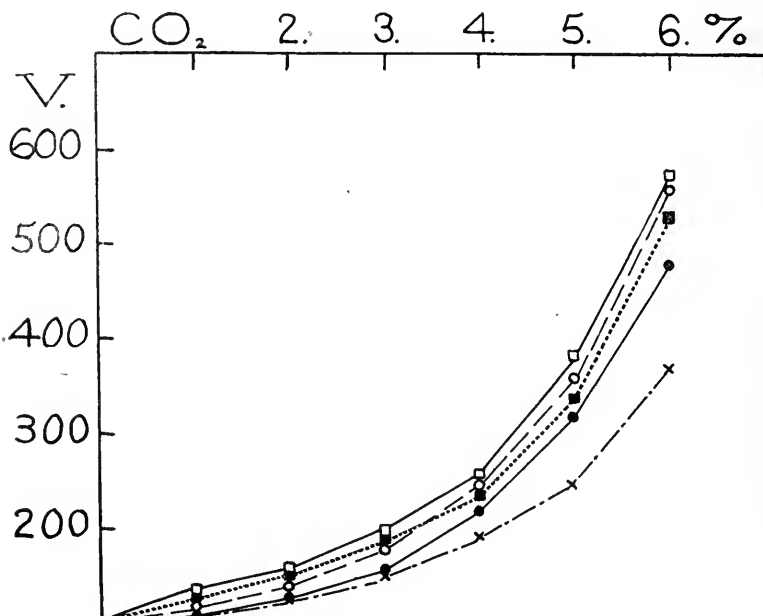

FIG. 1. AVERAGE CURVES FOR REACTION TO INCREASING AMOUNTS OF CO_2 IN THE INSPIRED AIR

Subject G. P. G. No caffeine; shown by solid line and dots. Same after caffeine; by broken line and circles. J. H. M. no caffeine; by dotted line and black squares. Same after caffeine; solid line and hollow squares. An average curve of three experiments done after taking sodium bicarbonate, shown by broken line and crosses. V, Ventilation coefficient.

strated more strikingly in text figure 1 in which the average figures of each subject's normal reactions and his reactions after caffeine have been plotted. The value of these averages is not very great inasmuch as there is more difference in the individual curves comprising each average curve than there is in the several

TABLE I

Data on all experiments

EXPERIMENT NUMBER	DATE	TIME	CAFFEINE grams	min.	WHILE BREATHING ATMOSPHERIC AIR Total ventilation liters per min.	Rate of respiration	Volume per respiration cc.	WHILE BREATHING CARBON DIOXID 5 PER CENT Total ventilation liters per min.	Rate of respiration	Volume per respiration cc.	MAXIMUM VENTILATION ATTAINED. LITERS PER MINUTE	PER CENT OF CARBON DIOXID IN INSPIRED AIR WHEN MAXIMUM VEN-TILATION WAS ATTAINED	1 Per cent	2 Per cent	3 Per cent	4 Per cent	5 Per cent	6 Per cent
					Experiments with G. P. G.													
2	December 23	10.00 a.m.	0.00		6.30	16.5	380	19.10	21.5	890	31.05	6.32	107	123	158	216	303	445
3	December 24	10.00 a.m.	0.00		7.45	19.0	390	17.35	19.0	915	34.70	6.50	103	115	137	175	233	340
15A	August 21	2.15 p.m.	0.00		7.60	15.5	490	31.65	24.5	1295	47.50	5.94	117	135	165	257	417	640
15B	August 21	3.20 p.m.	0.32	35	10.60	23.0	460	37.10	28.5	1300	83.00	7.07	112	133	170	233	350	508
15C	August 21	4.40 p.m.	0.32	115	8.38	23.5	355	33.10	29.5	1120	59.70	6.10	105	120	158	238	395	678
9	January 16	10.15 a.m.	0.60	120	3.42	20.0	420	28.90	27.5	1050	42.90	6.23	124	153	200	255	343	470
13	February 14	8.45 a.m.	0.60	90	7.94	20.5	385	26.75	27.0	990	40.00	5.76	110	135	177	238	337	
					Experiments with J. H. M.													
5	January 1	10.00 a.m.	0.00		5.64	9.5	595	17.80	17.5	1015	33.15	6.99	117	150	192	240	316	433
7	January 12	11.00 a.m.	0.00		7.77	16.0	485	22.30	23.5	950	62.00	7.37	105	117	141	193	287	450
16A	August 25	2.30 p.m.	0.00		7.10	11.5	615	21.65	23.0	940	65.00	6.28	147	128	148	196	305	700
17A	September 16	2.25 p.m.	0.00		5.40	10.0	540	23.75	22.5	1050	52.40	5.60	110	195	242	300	440	
16B	August 25	3.40 p.m.	0.20	30	7.88	11.5	685	24.80	19.5	1270	62.10	6.50	130	136	173	227	315	515
17B	September 16	3.50 p.m.	0.65	40	7.75	13.0	595	32.15	28.0	1150	60.00	5.50	130	158	186	233	415	
17C	September 16	5.10 p.m.	0.65	120	6.22	10.0	620	27.05	26.5	1020	65.40	5.70	130	160	205	270	435	
11	January 24	9.00 a.m.	0.75	60	7.38	15.0	490	27.80	24.0	1160	45.00	6.00	137	175	223	285	377	610
14	February 14	10.00 a.m.	0.75	120	7.52	12.0	625	26.30	23.0	1140	26.30	5.00	120	153	192	245	350	

average curves.   Nevertheless it is fair to say that no greater change in the reaction to carbon dioxide results from the taking of caffeine than occurs spontaneously.

Why there is so much variation in the normal curves is not clear.   It is quite possible that the sensitivity of the respiratory center varies from day to day and at different times during the same day.   Experiments 15, 16 and 17, in which curves were secured before and after the taking of caffeine without the subject's leaving the couch, undoubtedly form a more ideal basis of comparison.   An inspection of the figures of these experiments, however, will fail to show any consistent change after taking the caffeine.   In text figure 2 the three curves obtained in experiment 17 are shown.   In this case the actual points obtained have been plotted instead of the coefficients read off from the most probable curves at the even percentages.   One of these curves was obtained before the subject had taken caffeine, two were after.   The three are exceedingly similar.

The question arises whether any drugs can alter the reaction. This can be answered in the affirmative in regard to depression at least, for Newburgh, Means and Porter (9) found a definite flattening in the curve in cats under the influence of urethane and in dogs under ether.   It seems most probable that these results were due to an effect on the center, though of course a change in the reaction of the blood will also alter the reaction, as has been very beautifully shown by Peabody (8), in the marked increase in rate of reaction to carbon dioxide in cases of acidosis. The opposite of Peabody's results is found in three curves which we obtained after taking sodium bicarbonate.   The figures of these are given in table 2 and the average of the three is indicated in text figure 1.

In regard to the mechanism whereby the increase in ventilation to increasing amounts of carbon dioxide is accomplished, we can also say that caffeine has no observable effect.   An increase in ventilation can be produced by either an increase in the rate or in the depth of respiration.   It is the function of the respiratory center to regulate lung ventilation as was shown in the classical work of Haldane and Priestley (11).   The character

of the respiration, on the other hand, that is, the relation of rate to depth, is probably controlled by some other mechanism. If we represent the depth of respiration (volume per respiration)
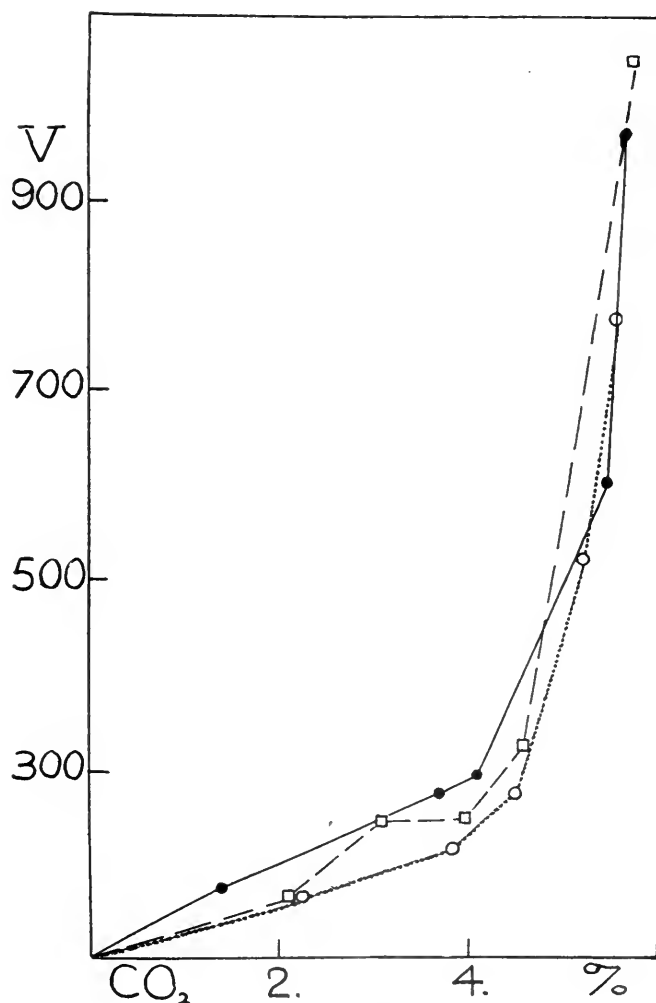
Fig. 2. Experiment 17.

Subject J. H. M. Curve *A*, before caffeine, shown by solid line and dots. Curve *B*, after caffeine, by dotted line and circles. Curve *C*, after caffeine, by broken line and squares.

by D and the rate by R and then calculate the value of the ratio $\frac{D}{R}$, we get the results shown in table 3. These figures indicate that while G. P. G. accomplishes his increase in ventilation chiefly by an increase in depth, J. H. M. accomplishes his by an in-

TABLE 2

Reaction to carbon dioxide after taking sodium bicarbonate

SUBJECT	VENTILATION COEFFICIENT WHEN PERCENTAGE OF CARBON DIOXID IN INSPIRED AIR WAS AS GIVEN BELOW						REMARKS
	1	2	3	4	5	6	
G.P.G.	110	123	150	187	240	326	7 grams sodium bicarbonate 1¼ hours before experiment.
G.P.G.	105	114	132	170	237	380	7 grams sodium bicarbonate ½ hour before experiment.
J.H.M.	112	131	160	195	245	383	10 grams sodium bicarbonate ½ hour before experiment.

NOTE. In each instance the urine secreted during the time the experiment was in progress, was alkaline to litmus.

TABLE 3

The ratio of depth of respiration to rate $\left(\dfrac{D}{R}\right)$ Averages before and after caffeine with each subject

SUBJECT		WHILE BREATHING ATMOSPHORIC AIR	WHILE BREATHING 5 PER CENT CARBON DIOXID
G. P. G.	Without caffeine	24.7	47.7
G. P G.	With caffeine	18.7	39.7
J. H. M.	Without caffeine	47.7	45.8
J. H. M.	With caffeine	49.0	49.0

crease in rate proportional to that in depth. With G. P. G., while the ratio $\frac{D}{R}$ is greater when he is breathing 5 per cent carbon dioxide than when breathing air, it is not altered to any striking degree by taking caffeine, and with J. H. M. the ratio $\frac{D}{R}$ is affected neither by an increase in CO_2 nor by caffeine.

The only positive finding in this research is confirmatory of the findings in the earlier papers, namely, that the total ventilation is increased by caffeine. This is well shown in experiments 15, 16 and 17. It is known that caffeine increases the metabolism. This was shown in the papers already cited, and also more recently in calorimeter experiments carried out at the Russell Sage Institute of Pathology by Means, Aub and Du Bois (6). The increase in ventilation, however, cannot be explained entirely as the result of increased CO_2 elimination for Higgins and Means (2) found that the tension of the alveolar CO_2 fell, which result could not have been due merely to increased elimination.

It has been said that caffeine reduces the fatiguability of muscles. Also Peabody and Wentworth (12) have shown a definite relation between dyspnoea and vital capacity of the lungs and concluded from that that the symptom dyspnoea was reached when the limitation of the lungs as a bellows began to be approached. The efficiency of the lungs as a bellows must depend in part on the state of the muscles of respiration; therefore we thought it conceivable that caffeine, through its effect on the musculature, might increase the efficiency of the lung as a bellows, or postpone the advent of dyspnoea by reducing the fatiguability of the respiratory musculature. Our experiments were all discontinued when the dyspnoea became distressing. When the subject had had "enough" he gave a signal to the experimenter to have the observation stopped. The total ventilation and the percentage of CO_2 breathed when the observations were stopped are all given in table 1. There seems to be no striking difference in the caffeine and normal curves as regards the onset of distressing dyspnoea with the single exception of experiment 15 B, which was the highest ventilation observed and which also was after caffeine.

<div align="center">CONCLUSION</div>

No significant change was found in the rate of reaction to carbon dioxide as a result of taking various doses (small and large) of caffeine alkaloid, by mouth, in two normal human subjects.

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ON CERTAIN ANTAGONISTS OF PILOCARPINE

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When a frog's heart is perfused in situ with a solution of pilocarpine not sufficiently concentrated to stop the heart but still active, a double effect is produced—the rate of the beat is considerably slowed and the force of the systolic contraction is diminished. Of these two actions, the systolic usually precedes the slowing by a brief interval. If the perfusion is prolonged to one to two hours both the diminution of the systole and the slowing remain with little or no alteration the whole time (fig. 1).
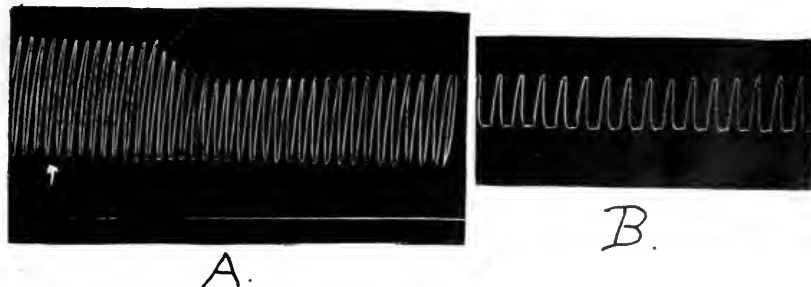
FIG. 1. CONTINUOUS PERFUSION WITH PILOCARPINE

A, At arrow perfusion with pilocarpine 0.01 per cent begins; *B*, continuation of perfusion, tracing begins fifty minutes later than end of *A*.

As regards contraction, the auricle is more strongly affected than the ventricle, so that the latter often goes on beating regularly and fairly well whilst the former is almost or quite still. The slowing is diastolic. There is usually no evidence of heart block but under a prolonged pilocarpine perfusion it occasionally develops.

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These effects correspond very closely with those which can be obtained by stimulation of the vago-sympathetic nerve. Hence it is generally considered that pilocarpine acts by stimulating the endings of the heart vagus, and this view is confirmed by the fact that atropine which paralyses vagal endings cuts out both effects of pilocarpine. This double action of pilocarpine is of considerable interest because, as is partly known and will be shown in extenso below, one of the two effects can be antagonized by certain drugs whilst the other remains intact.

In the following communication a closer analysis of this fact is attempted. The method adopted was to try the action of various drugs in solution with pilocarpine and so to see how far they would act as antagonists. The investigation was confined to frog's hearts. All frogs were pithed. The hearts were perfused in situ from the vena cava or from the sinus, the perfusion fluid flowing out by the cut aorta. A constant pressure was maintained.

Pilocarpine-atropine. It should be noted that the effect of the same percentage of pilocarpine varies somewhat with different hearts; as a rule 0.001 per cent causes distinct diminution of systole followed by more or less slowing. With 0.005 per cent the slowing and the diminution of systole begin almost immediately. Sometimes though not always 0.02 per cent is sufficient to stop the heart (fig. 2). With small percentages of pilocarpine the systolic effect usually sets in sooner than the slowing (fig. 2, *a*); this may perhaps be taken to indicate that pilocarpine attacks the vagal mechanism in the heart at two points, one of which is more concerned with the force of the systole (inotrope), the other with the heart rate (chronotrope). In most cases when pilocarpine with atropine is perfused after pilocarpine, the slowing and the diminution of systole begin to disappear almost simultaneously but it is occasionally possible to find a dose of atropine which distinctly restores the systole first.

Pilocarpine-strontium. Strontium increases the efficiency of the heart by making the systole more complete; if the dose is

carefully regulated the relaxation in diastole is not diminished but the heart is more or less slowed because of the tendency to remain in systole and the consequent slowness of the relaxation. So far as the systolic effect of pilocarpine is concerned there is a very marked antagonism between the two drugs, whilst the
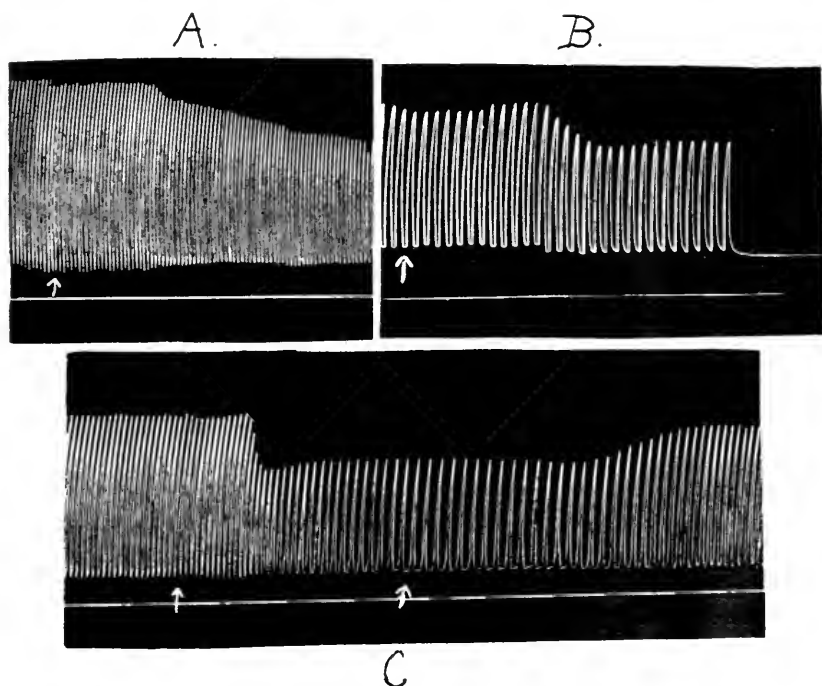
FIG. 2. EFFECTS OF VARIOUS CONCENTRATIONS OF PILOCARPINE

A, Normal heart, at arrow perfusion with pilocarpine 0.001 per cent begins; B, normal heart, at arrow perfusion with pilocarpine 0.02 per cent begins; C, normal heart, at first arrow perfusion with pilocarpine 0.005 per cent begins, at second arrow belladonna added to pilocarpine.

slowing which pilocarpine causes is quite unaffected by strontium (fig. 3.)

It will be noted that, as shown in figure 1, even after an hour's perfusion there is no change in the rate which pilocarpine had set up. Strontium acts upon the heart muscle and the slowing which it produces is not affected by atropine. It appears that

pilocarpine acting through the vagal endings produces in the heart muscle a condition in which there is a less effective response in systole. This condition is reversed by strontium and the systole becomes normal or is increased. This result seems to suggest that the negative inotrope action of pilocarpine is distinct from the negative chronotrope effect, i.e., that the changes causing the slowing are distinct from those causing diminution of systole and that there are in fact two functionally differentiated divisions of the vagus nerve in the frog's heart.
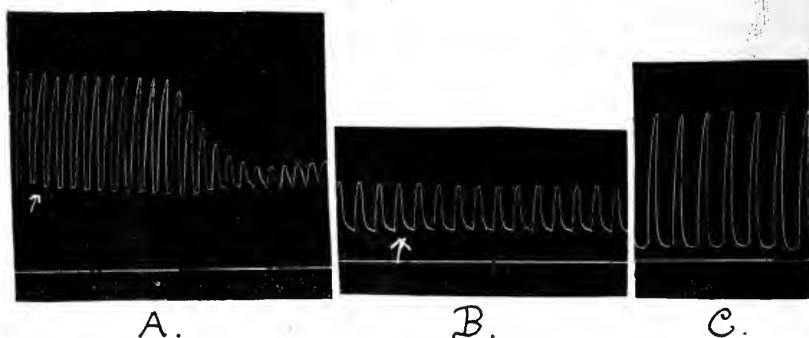
FIG. 3. PARTIAL ANTAGONISTIC EFFECT OF STRONTIUM

A, Normal heart, at arrow perfusion with pilocarpine 0.01 per cent begins; B, perfusion continued, tracing begins six minutes later than end of A, at arrow perfusion changed to pilocarpine 0.01 per cent + SrCl₂ 0.05 per cent; C, perfusion continued, tracing begins six minutes later than end of B.

Pilocarpine-digitalis. That an antagonism exists between pilocarpine and digitalis is already established; Cushny (1) says that digitalin and its allies remove the standstill (from pilocarpine) by increasing the irritability of the muscle until the inhibition can no longer hold the heart in check, though the rhythm remains slow. If digitalis is perfused alone for some time and then together with pilocarpine the systolic effect of pilocarpine may be completely prevented (fig. 4).

On the other hand if the perfusion is commenced with both together there is first a marked pilocarpine action on systole and rate even going to prolonged stoppage of the heart from

which however gradual restoration of systole occurs though the rate remains slow (fig. 5).

The tracing (fig. 6) shows marked slowing under combined perfusion with pilocarpine and digitalis probably due to pilocarpine, since the slowing from digitalis takes more time to develop. It is also evident that pilocarpine acts more quickly than digitalis, and we have again an indication that the slowing and the systolic effects of pilocarpine are produced from

A.   B.   C.

FIG. 4. PROPHYLACTIC EFFECT OF DIGITALIS

A, Normal heart, at arrow perfusion with tincture digitalis 0.4 per cent begins; B, perfusion continued, tracing begins two minutes later than end of A; C, perfusion continued, tracing begins seven minutes later than end of B, at arrow perfusion changed to pilocarpine 0.01 per cent + tincture digitalis 0.4 per cent.

different points in the persistence of the slowing after complete restoration of systole (fig. 6, B).

Pilocarpine—agaricine. McCartney has called attention to the similarity in the cardiac actions of agaricine and digitalis and also to the fact that agaricine antagonizes the systolic effect of pilocarpine.

Pilocarpine-caffeine. When the heart is perfused first with caffeine and then with caffeine and pilocarpine together the pilocarpine fails to produce any obvious effect either on the heart rate or on the efficiency of the systole (fig. 7).

If the perfusion is commenced with pilocarpine and caffeine together the caffeine concentration being fairly strong, only the caffeine action on systole is produced (fig. 8).

If the combined perfusion fluid contains rather less caffeine there is a brief effect of pilocarpine upon systole and then the
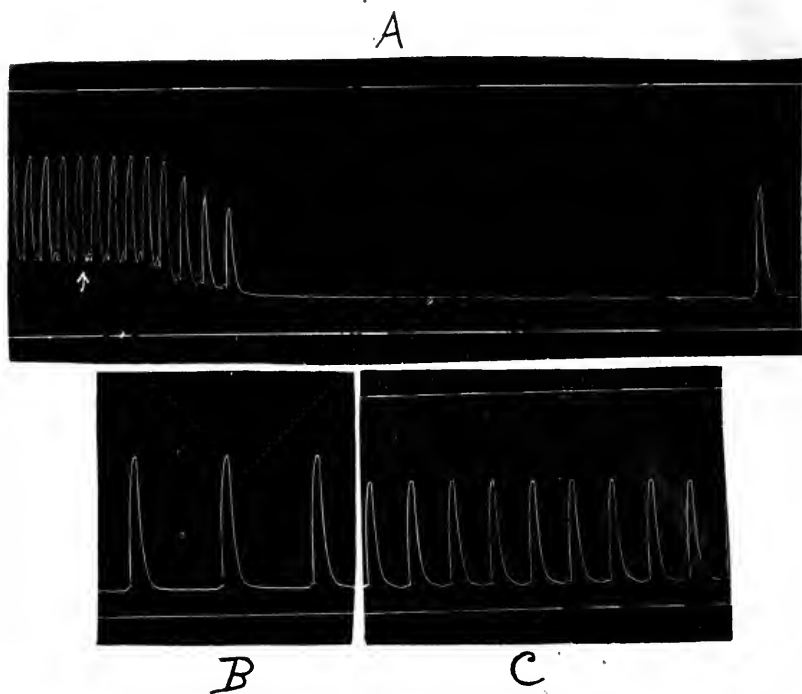
FIG. 5. PARTIAL ANTAGONISM OF DIGITALIS

A, Normal heart, at arrow combined perfusion with pilocarpine 0.01 per cent + tincture digitalis 0.4 per cent begins; B, perfusion continued, tracing begins three minutes later than end of A; C, perfusion continued, tracing begins four minutes later than end of B.

systole is more or less increased and the heart goes on beating strongly without the rate having been affected at all (fig. 9).

If the perfusion is begun with pilocarpine alone and after the inotrope and chronotrope effects have appeared caffeine is added to the perfusion fluid both the pilocarpine effects disappear and the heart is restored (fig. 10). It is evident from these

results that caffeine is able to antagonize pilocarpine almost as effectively as atropine.
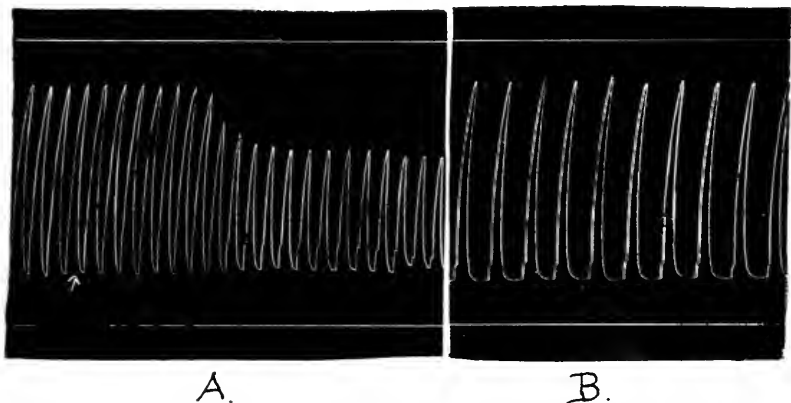
FIG. 6. PARTIAL ANTAGONISM OF DIGITALIS

A, Normal heart, at arrow combined perfusion with tincture digitalis 0.4 per cent + pilocarpine 0.01 per cent begins; *B*, perfusion continued, tracing begins four minutes later than end of *A*.
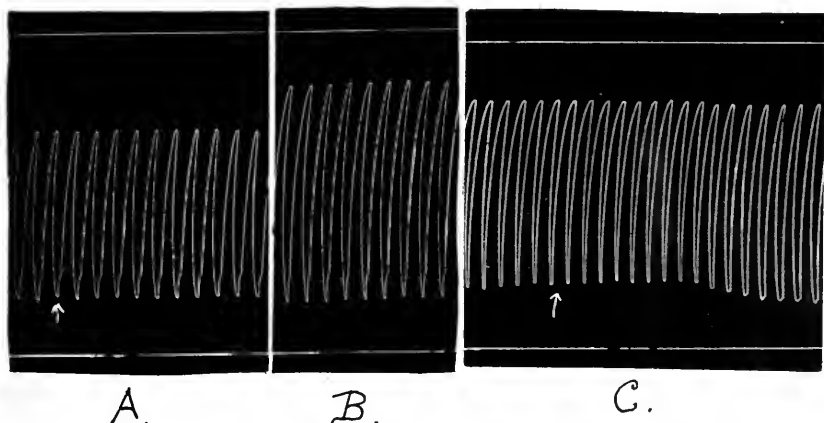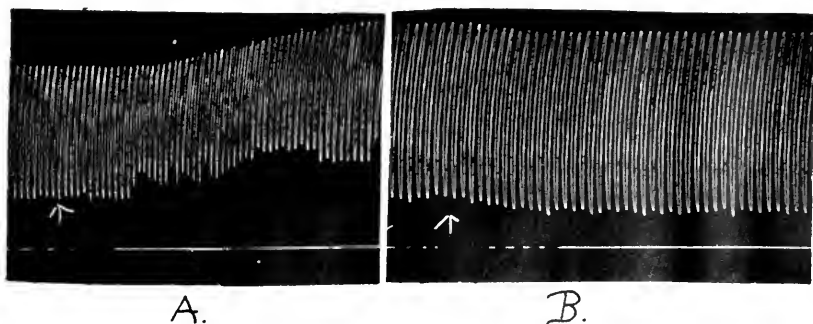

FIG. 7. PROPHYLACTIC EFFECT OF CAFFEINE

A, Normal heart, at arrow perfusion with caffeine 0.25 per cent begins; *B*, perfusion continued, tracing begins four minutes later than end of *A*, at arrow perfusion changed to pilocarpine 0.02 per cent + caffeine 0.25 per cent.

Pilocarpine-adrenalin. If the perfusion is commenced with pilocarpine and adrenalin together there is a brief pilocarpine diminution of systole but rapid recovery takes place and the

heart goes on beating with improved systole and scarcely a trace of slowing (fig. 11). If the perfusion is commenced with pilocarpine alone and after the inotrope and chronotrope effects
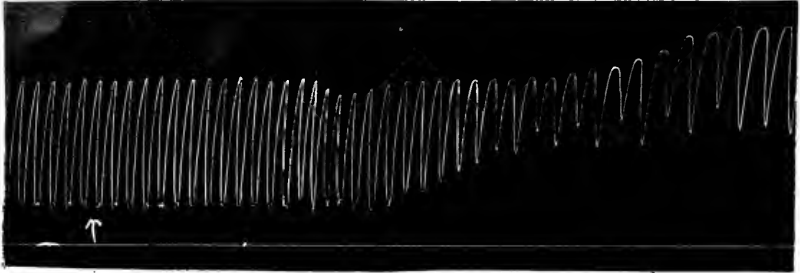
FIG. 8. ANTAGONIZING EFFECT OF CAFFEINE IN COMBINED PERFUSION

Normal heart, at arrow perfusion with pilocarpine 0.01 per cent + caffeine 0.5 per cent begins.
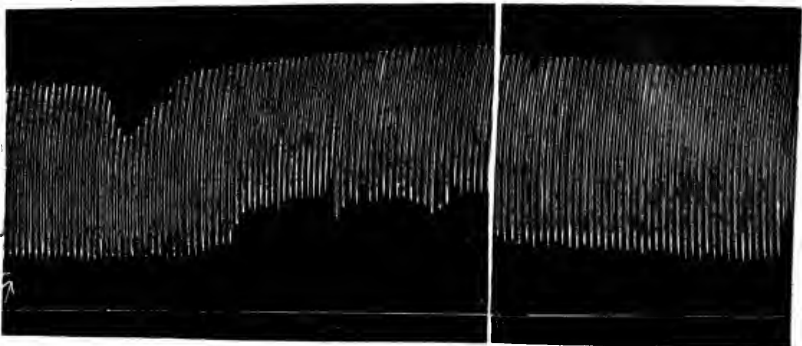
FIG. 9. ANTAGONIZING EFFECT OF CAFFEINE IN COMBINED PERFUSION (MORE PILOCARPINE AND LESS CAFFEINE THAN IN FIGURE 8)

A, Normal heart, at arrow perfusion with pilocarpine 0.02 per cent + caffeine 0.25 per cent begins; B, combined perfusion continued, tracing begins four minutes later than end of A.

have appeared adrenalin is added to the perfusing fluid the systole is rapidly restored and the heart is quickened but there is still some slowing as compared with the normal (fig. 12).

If adrenalin is perfused first and followed by pilocarpine alone the pilocarpine has very little effect either upon the heart rate or upon the systole.   It is obvious therefore that adrenalin antagonizes the heart action of pilocarpine very effectively.

A.   B.   C.

FIG. 10. ANTAGONIZING EFFECT OF CAFFEINE IN COMBINED PERFUSION AFTER PILOCARPINE ALONE

A, Normal heart, at arrow perfusion with pilocarpine 0.02 per cent begins; B, perfusion continued, tracing begins two minutes later than end of A, at arrow change to pilocarpine 0.02 per cent + caffeine 0.25 per cent; C, combined perfusion continued, tracing begins five minutes later than end of B.

A.   B.

FIG. 11. ANTAGONISM OF ADRENALIN IN COMBINED PERFUSION

A, Normal heart, at arrow perfusion with pilocarpine 0.02 per cent + adrenalin 1: 25000 begins; B, perfusion continued, tracing begins four minutes later than end of A, at arrow change to pilocarpine 0.02 per cent alone—prophylactic effect of previous adrenalin.

Pilocarpine-saponine. When the perfusion is commenced with saponine and continued with pilocarpine and saponine together the chronotrope action of pilocarpine is quickly developed whilst the inotrope effect is slight (fig. 14).

When the perfusion is commenced with pilocarpine and sap-
onine together both effects of pilocarpine are developed but on
continuing the perfusion the force of the systole increases again
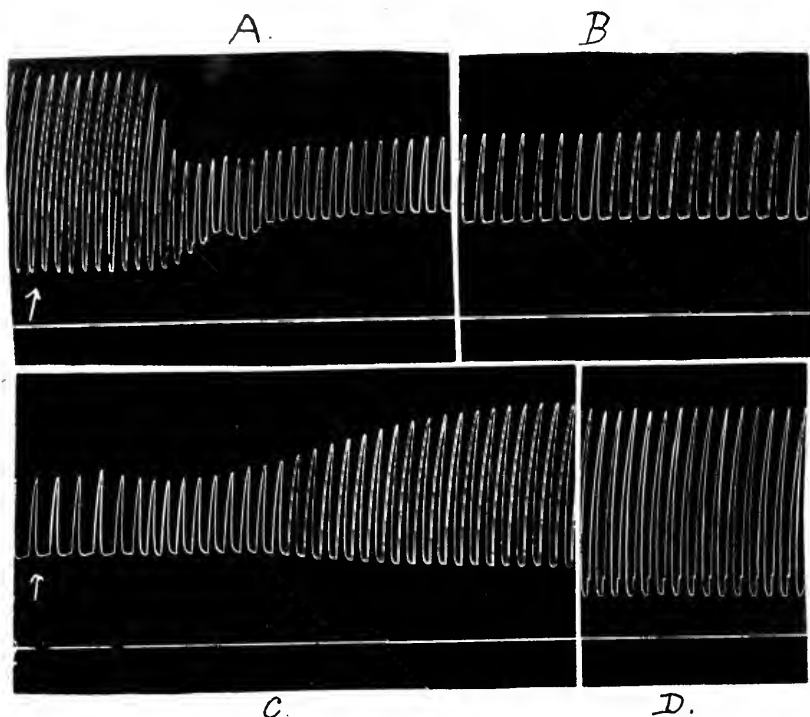
FIG. 12. ANTAGONIZING EFFECT OF ADRENALIN IN COMBINED PERFUSION AFTER
PILOCARPINE ALONE

A, Normal heart, at arrow perfusion with pilocarpine 0.02 per cent begins;
B, perfusion continued, tracing begins two minutes later than end of A; C, per-
fusion continued, tracing begins one minute later than end of B, at arrow change
to pilocarpine 0.02 per cent + adrenalin 1:25000; D, perfusion continued, tracing
begins three minutes later than end of C.

whilst the slowing remains temporarily unaltered, showing that
under these conditions saponine is able to antagonize the negative
inotrope action of pilocarpine but leaves the negative chrono-
trope action for a time in full strength, later on however the

negative chronotrope effect of pilocarpine becomes gradually less (fig. 15).   When the perfusion is commenced with pilocar-
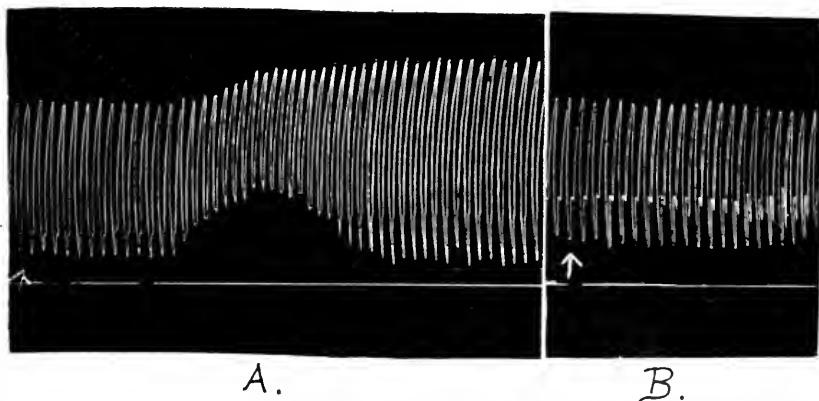
FIG. 13. PROPHYLACTIC EFFECT OF ADRENALIN

A, Normal heart, at arrow perfusion with adrenalin 1:25000 begins; *B*, perfusion continued, tracing begins seven minutes later than end of *A*; at arrow change to pilocarpine 0.02 per cent.

FIG. 14. PARTIAL PROPHYLACTIC EFFECT OF SAPONINE

A, Normal heart, at arrow perfusion with saponine 0.003 per cent begins; *B*, perfusion continued, tracing begins nine minutes later than end of *A*; at arrow change to pilocarpine 0.01 per cent + saponine 0.003 per cent.

pine alone and continued with pilocarpine and saponine together both effects of pilocarpine gradually disappear (fig. 16).
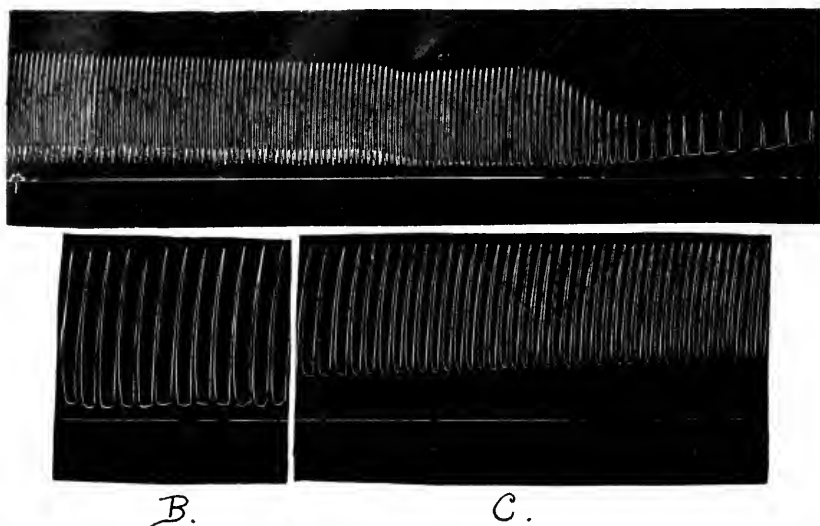
FIG. 15. PARTIAL ANTAGONISM OF SAPONINE IN COMBINED PERFUSION

A, Normal heart, at arrow perfusion with pilocarpine 0.01 per cent + sapo-
nine 0.003 per cent begins (note long incubation before pilocarpine begins to act);
B, perfusion continued, tracing begins twelve minutes later than end of A; C,
perfusion continued, tracing begins five minutes later than end of B.
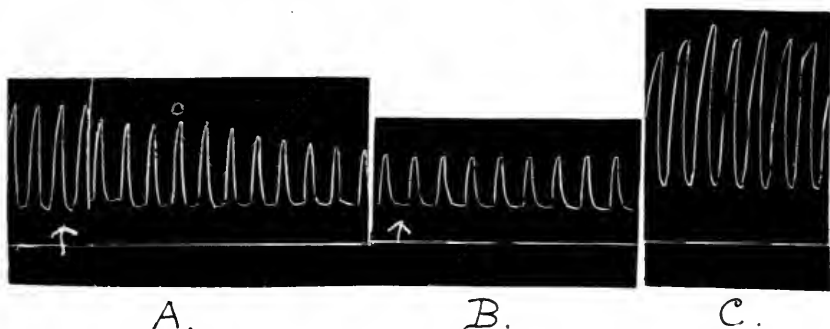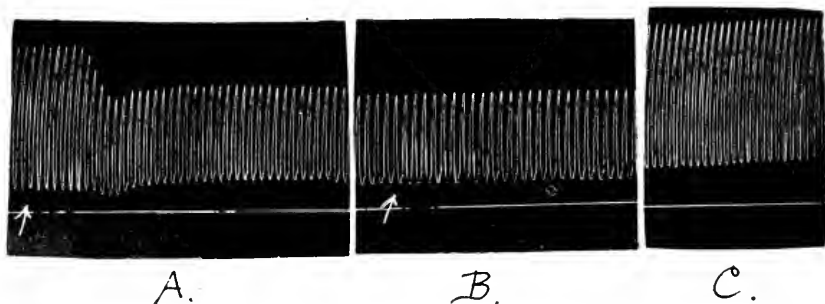

FIG. 16. ANTAGONISM OF SAPONINE IN COMBINED PERFUSION AFTER PILOCARPINE

A, Normal heart, at arrow perfusion with pilocarpine 0.01 per cent begins;
B, perfusion continued, tracing begins three minutes later than end of A; at
arrow change to pilocarpine 0.01 per cent + saponine 0.003 per cent; C, perfu-
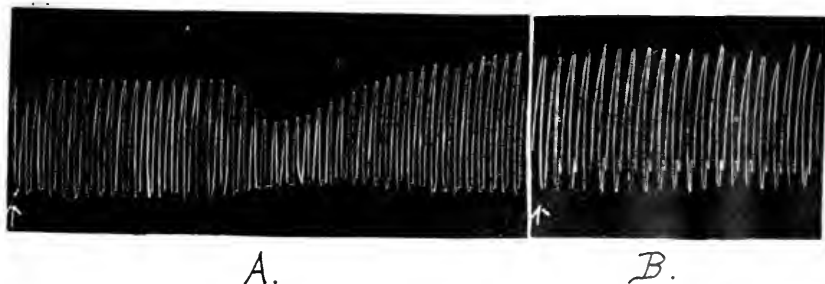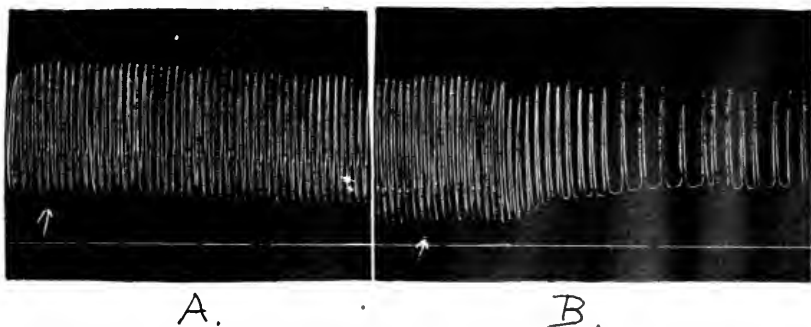sion continued, tracing begins ten minutes later than end of B (note the commenc-
ing rise of tone due to saponine).

DISCUSSION

First with reference to the antagonism between pilocarpine and atropine, it is probably justifiable to say that pilocarpine produces physico-chemical changes closely resembling those which follow stimulation of the vagus and that these changes are reversed by atropine. Naturally in comparing results account has to be taken of the fact that whilst on the cessation of the vagal stimulation a fairly rapid restoration takes place the condition produced by pilocarpine can only be gradually reversed since the removal or destruction of the pilocarpine takes time.

The chronotrope and inotrope effects of pilocarpine are entirely removed by atropine though usually the latter goes first if the atropine concentration is low; moreover it is possible to find a dose of atropine which will remove the systolic depression brought about by pilocarpine without at first affecting the slowing. This fact would seem to indicate that the vagal fibers controlling the heart rate are not identical with those which affect the systole. Hofmann's (3) researches go to show that this is in fact the case. He says that the vagal fibers in the frog's heart are divided into two sets, the one affecting the efficiency of the ventricular systole, negative inotrope, the other the heart rate, negative chronotrope. The former set are contained exclusively in the nerves which run over the septum, the other set are distributed in the auricular wall. When the heart is rhythmically excited by an electric current the effect of stimulating the vagus is to reduce the systole, if however the septal nerves are first cut no diminution of systole occurs. Further, in the frog's heart excitement of the peripheral stump of the cut septal nerves has no negative chronotrope effect upon the ventricle.

In the light of Hofmann's results it is possible to offer a partial explanation of the antagonism between pilocarpine and atropine as also of that between pilocarpine and the other substances investigated in the present research.

Pilocarpine excites vagal endings irrespective apparently of their functions, it has therefore a negative chronotrope and a

negative inotrope action upon the frog's heart, both groups of vagal fibers being affected, and these actions are both reversed by atropine because it paralyses vagal endings. Strontium in certain concentration increases the efficiency of the ventricular contraction and to some extent slows the heart but the slowing is due to prolonged contraction and delay in relaxation, is not diastolic and not affected by atropine. In fact strontium acts upon muscle and not upon nerve. Strontium antagonizes the negative inotrope effect of pilocarpine because it increases the ventricular efficiency which pilocarpine, by stimulating the vagal endings, impairs. On the other hand strontium has no effect upon the pace-regulating mechanism itself nor does it act upon the vagus and hence does not antagonise the negative chronotrope action of pilocarpine. The same explanation applies to the partial antagonism between pilocarpine and digitalis. In the frog's heart digitalis is essentially a muscle poison, its slowing action is not prevented by atropine. It increases the efficiency of the ventricular systole and so antagonizes the negative inotrope action of pilocarpine but it neither affects the pace making mechanism directly nor the vagus and so the negative chronotrope action of pilocarpine remains unhindered.

When the fresh heart is perfused with pilocarpine and digitalis together there is often a short negative inotrope pilocarpine effect which however quickly passes off; this may doubtless be attributed to the greater slowness with which digitalis is taken up by the heart muscle. On the other hand a preliminary perfusion with digitalis alone will often entirely prevent any negative inotrope effect of pilocarpine when the two drugs are afterwards perfused together.

The partial antagonism between pilocarpine and agaricine is of the same nature as that between pilocarpine and digitalis, and is explained in the same way since, as McCartney has pointed out, agaricine must be looked upon as a muscle poison much resembling digitalis.

The antagonism between pilocarpine and caffeine is of considerable interest because it is very nearly if not quite complete, almost as complete as that between pilocarpine and atropine. As can be seen in figure 7 a few minutes preliminary perfusion

with caffeine suffices to prevent entirely any pilocarpine action when the two drugs are afterwards perfused together. If the fresh heart is perfused with a mixture of pilocarpine and caffeine there is only a very brief inotrope action and no negative chronotrope effect whatever. In the frog's heart caffeine stimulates the excito-motor mechanism and at the same time affects the heart muscle increasing the intensity of the ventricular contraction; it has no action on the vagal endings. It is by means of this double action apparently that caffeine is able to antagonize both the negative inotrope and the negative chronotrope effects of pilocarpine. The fact that this particular substance is so very efficient an antagonist to pilocarpine would seem strongly to support the explanation offered above in the cases of strontium, digitalis and agaricine, namely that there are two sets of vagal fibers with different functions in the frog's heart and that the effects of their stimulation may be antagonized singly.

The results obtained with caffeine suggest that in similar conditions adrenalin would also antagonize pilocarpine and as shown above this was in fact found to be the case. A preliminary perfusion with adrenalin prevents both the chronotrope and the inotrope actions of pilocarpine. When pilocarpine and adrenalin are perfused first together, there is only a very short pilocarpine effect on systole, afterwards pilocarpine alone has no effect at all (fig. 13). The sympathetic is the physiological antagonist of the vagus in the heart, adrenalin stimulates the sympathetic endings and therefore in suitably regulated doses antagonizes pilocarpine.

The action of saponine upon the frog's heart has certain similarities with that of digitalis, for it causes in moderate concentration some increase in systole and more or less rise in tone. The action is apparently chiefly upon the heart muscle. An antagonism to the inotrope effect of pilocarpine was therefore anticipated and in fact found (figs. 15 and 16); later on however under continued perfusion with pilocarpine and saponine together the chronotrope effect also disappeared, this latter observation awaits for the moment an explanation. The tracings afford striking evidence of the fundamental distinction between the two activities of pilocarpine, e.g., in figure 15 where the

inotrope effect is completely antagonized whilst the negative chronotrope action continues unimpaired.

The net result of these experiments is to make it clear that the chronotrope and inotrope actions of pilocarpine are fundamentally distinct. They are both vagal effects but different groups of vagal fibers are concerned in their production and hence one action can be antagonized whilst the other remains intact.

SUMMARY

Stimulation of the vagus has two distinct effects upon the heart, a negative chronotrope and a negative inotrope. These separate functional activities are associated with different fibers of the vagus (Hofmann). Pilocarpine stimulates vagal endings whatever their function and has in consequence a negative chronotrope and a negative inotrope effect. Atropine paralyses vagal endings and hence entirely antagonizes the heart actions of pilocarpine. Strontium, digitalis, agaricine and saponine which, without affecting the vagus, have a positive inotrope action upon the frog's heart antagonize the negative inotrope action of pilocarpine but leave the negative chronotrope effect unimpaired. Adrenalin and caffeine antagonize both the actions of pilocarpine, not because they affect either of the two sets of vagal fibers but because they produce effects the reverse of those which stimulation of the two vagal groups brings about.

Considerable interest attaches to the fact that strontium, digitalis, etc., which act upon muscular tissue antagonize the pilocarpine (i.e., vagal) action on systole but do not antagonize the action (also vagal) upon the heart rate. It seems therefore obvious that the changes in the muscular tissue which accompany diminution of systole are not identical with those which are associated with change of rate, as otherwise both would be antagonized by strontium.

REFERENCES

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PHARMACOLOGICAL STUDIES OF THE IPECAC ALKALOIDS AND SOME SYNTHETIC DERIVATIVES OF CEPHAELINE

II. STUDIES ON EMETIC EFFECT AND IRRITANT ACTION

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A. EMETIC EFFECT

Both emetine and cephaeline are known to be emetic in small doses when taken by mouth. Since emetine is cephaeline methyl ether and is less emetic than cephaeline, it was of interest to determine the relative emetic power of some of the higher homologues of this series. The following ethers of cephaeline were prepared and their emetic action compared with that of cephaeline and emetine: ethyl, propyl, butyl, iso-amyl and allyl. Cats were chosen as suitable animals for these emetic tests. Full grown cats were fed milk about 7.30 a.m. At 10.00 a.m. each cat received 1 ounce of fresh raw beef, finely cut. Between 11.30 and 12.00 the alkaloidal salt was given in solution by stomach tube, the solution with the washings amounting to 20 cc. The animals were kept under observation until 5.30 p.m. The same animal could not be used satisfactorily oftener than once a week. After continued use the animals became more susceptible to the emetic effect of the drug. As different cats varied considerably as to the amount of drug required to produce emesis, it was necessary to give at least two of the alkaloids to the same animal in order to obtain a correct comparison. This difference in reaction and the increasing susceptibility on repeated dosage necessitated the use of a large

number of animals. In the experiments summarized here, fifty-six cats were used and the period of experimentation extended over several months. The following protocols illustrate the method pursued, but conclusions must be based on a series of such tests.

Protocols

ANIMAL	DRUG	DOSE PER CAT	RESULT
		gram	
92	Cephaeline hydrochloride....................	0.002	Vomited
	Cephaeline hydrochloride....................	0.002	Vomited
	Emetine hydrochloride......................	0.004	Did not vomit
	Emetine hydrochloride......................	0.004	Did not vomit
86	Cephaeline hydrochloride....................	0.002	Vomited
	Cephaeline hydrochloride....................	0.002	Did not vomit
	Cephaeline hydrochloride..............,.....	0.002	Did not vomit
	Emetine hydrochloride......................	0.005	Vomited
	Emetine hydrochloride......................	0.004	Did not vomit
	Emetine hydrochloride......................	0.004	Did not vomit
12	Cephaeline hydrochloride....................	0.004	Vomited
	Cephaeline hydrochloride....................	0.003	Did not vomit
	Cephaeline hydrochloride....................	0.003	Did not vomit
	Cephaeline hydrochloride....................	0.002	Did not vomit
	Cephaeline propyl ether hydrobromide........	0.016	Vomited
	Cephaeline propyl ether hydrobromide........	0.012	Did not vomit
	Cephaeline propyl ether hydrobromide........	0.010	Did not vomit
	Cephaeline ethyl ether hydrobromide.........	0.012	Vomited
	Cephaeline butyl ether hydrobromide....... .	0.012	Vomited
	Emetine hydrochloride.......................	0.010	Vomited
85	Emetine hydrochloride.......................	0.008	Did not vomit
	Emetine hydrochloride.......................	0.012	Vomited
	Cephaeline propyl ether phosphate...........	0.012	Did not vomit
	Cephaeline propyl ether phosphate...........	0.016	Did not vomit
	Cephaeline propyl ether phosphate...........	0.020	Vomited
13	Emetine hydrochloride....................	0.008	Did not vomit
	Emetine hydrochloride......................	0.010	Did not vomit
	Emetine hydrochloride......................	0.012	Vomited
	Cephaeline iso-amyl ether hydrobromide......	0.024	Did not vomit
	Cephaeline iso-amyl ether hydrobromide......	0.030	Did not vomit
	Cephaeline iso-amyl ether hydrobromide......	0.036	Vomited

Protocols—Continued

ANIMAL	DRUG	DOSE PER CAT	RESULT
		gram	
	Cephaeline allyl ether phosphate............	0.015	Did not vomit
	Cephaeline allyl ether phosphate............	0.020	Vomited
	Emetine phosphate...........................	0.010	Did not vomit
31	Emetine phosphate...........................	0.012	Vomited
	Psychotrine hydrochloride...................	0.075	Did not vomit
	Psychotrine hydrochloride...................	0.100	Vomited
	Pyschotrine hydrochloride...................	0.100	Did not vomit
	Pyschotrine hydrochloride...................	0.120	Did not vomit
	Psychotrine hydrochloride..................	0.130	Did not vomit

A total of 290 doses were given and from an analysis of the several experiments the following comparative figures were compiled, the emetic dose of cephaeline having been taken as 1.

Cephaeline hydrochloride.. 1.
Cephaeline methyl ether (emetine) hydrochloride................. 2.
Cephaeline ethyl ether hydrobromide............................. 2.4
Cephaeline propyl ether hydrobromide............................ . 3.6
Cephaeline propyl ether phosphate............................... 3.8
Cephaeline butyl ether hydrobromide............................. 3.6
Cephaeline iso-amyl ether hydrobromide.......................... 5.0
Cephaeline ailyl ether phosphate................................ 2.5

The results are only approximately correct at best, but they show that the emetic power of this series of compounds diminishes much in the same order as does their toxicity (1) and that emetine hydrochloride is about one-half as emetic as cephaeline hydrochloride and that cephaeline iso-amyl ether hydrobromide is only one-fifth as emetic as cephaeline hydrochloride.

It was noted that some salts of these cephaeline compounds were more emetic than others, and accordingly the hydroiodides of the methyl, propyl and iso-amyl derivatives were tried in a manner similar to that above detailed, with the exception that the alkaloidal salts were all given in capsules instead of in solution.

The following tables are two of a series of seven and demonstrate the greatly diminished emetic power of the difficultly soluble hydroiodides of the cephaeline propyl and iso-amyl

ethers. Emetine hydroiodide was not appreciably weaker as an emetic than emetine hydrochloride.

Cat 14; weight, 3086 grams

DATE	DRUG	DOSE PER CAT	RESULTS
		gram	
April 5, 1916	Cephaeline propyl ether hydroiodide	0.020	Vomited
April 12, 1916	Cephaeline propyl ether hydroiodide	0.015	Did not vomit
April 19, 1916	Cephaeline propyl ether hydroiodide	0.020	Vomited
April 27, 1916	Cephaeline propyl ether hydroiodide	0.015	Did not vomit
May 5, 1916	Emetine hydrochloride.............	0.010	Vomited
May 12, 1916	Emetine hydrochloride.............	0.007	Vomited
May 19, 1916	Emetine hydrochloride.............	0.005	Did not vomit
May 26, 1916	Emetine hydrochloride.............	0.005	Vomited
June 2, 1916	Emetine hydroiodide..............	0.007	Vomited

Cat 18; weight, 2484 grams

DATE	DRUG	DOSE PER CAT	RESULTS
March 30, 1916	Cephaeline iso-amyl ether hydro-iodide..........................	0.040	Did not vomit
April 14, 1916	Cephaeline iso-amyl ether hydro-iodide..........................	0.050	Did not vomit
April 21, 1916	Cephaeline iso-amyl ether hydro-iodide..........................	0.060	Did not vomit
April 28, 1916	Cephaeline iso-amyl ether hydro-iodide..........................	0.060	Did not vomit
May 5, 1916	Cephaline iso-amyl ether hydro-iodide..........................	0.065	Did not vomit
May 12, 1916	Cephaeline iso-amyl ether hydro-iodide..........................	0.070	Did not vomit
May 19, 1916	Cephaeline iso-amyl ether hydro-iodide..........................	0.075	Did not vomit
May 26, 1916	Emetine hydrochloride.............	0.010	Did not vomit
June 2, 1916	Emetine hydrochloride.............	0.012	Vomited
June 15, 1916	Cephaeline iso-amyl ether hydro-iodide..........................	0.080	Did not vomit
June 23, 1916	Cephaeline iso-amyl ether hydro-iodide..........................	0.085	Did not vomit
July 7, 1916	Cephaeline iso-amyl ether hydro-iodide..........................	0.090	Did not vomit
July 21, 1916	Cephaeline iso-amyl ether hydro-iodide..........................	0.095	Vomited

A summary of the results with these seven cats is given below.

CAT	DRUG	EMETIC DOSE	DRUG	EMETIC DOSE
		mgm.		*mgm.*
14	Cephaeline propyl ether hydroiodide	15–20	Emetine hydrochloride	5
17	Cephaeline propyl ether hydroiodide	35–40	Emetine hydrochloride	10–12
19	Cephaeline propyl ether hydroiodide	35–40	Emetine hydrochloride	10–12
15	Cephaeline iso-amyl ether hydroiodide..........................	55–60	Emetine hydrochloride	7–10
16	Cephaeline iso-amyl ether hydroiodide..........................	75–80	Emetine hydrochloride	10–12
18	Cephaeline iso-amyl ether hydroiodide..........................	90–95	Emetine hydrochloride	10–12
20	Cephaeline iso-amyl ether hydroiodide..........................	60–65	Emetine hydrochloride	12–15

From these tests made on seven animals, cephaeline propyl ether hydroiodide is not more than one-third as emetic for cats as is emetine hydrochloride, and cephaeline iso-amyl ether hydroiodide is not more than one-sixth as emetic as emetine hydrochloride or one-twelfth as emetic as cephaeline hydrochloride, the mother substance.

A few tests on persons showed an individual variation in susceptibility to the ipecac alkaloids. As a rule one-eighth grain of cephaeline hydrochloride and one-fourth grain of emetine hydrochloride proved emetic. One-sixth grain of cephaeline iso-amyl ether hydrochloride caused slight nausea but no emesis in two persons and no noticeable effect in four others; one-third grain caused slight nausea in one case and no effect in another, and one person, a boy of sixteen years, took one-sixth grain three times a day for fourteen days without any symptoms of vomiting or nausea. The cephaeline iso-amyl ether hydroiodide was given in single doses of three-fourths of a grain without producing nausea. One person took one-fourth grain of this hydroiodide three times a day for fourteen days without nausea resulting. Larger doses than the above were not given.

The irritant action of emetine hydrochloride when injected hypodermatically has been noted by all who have used this alkaloidal salt. Its irritant effect has also been evident where accidentally solutions of it have come in contact with the conjunctiva or with the mucous membrane of the oral cavity. Furthermore, workers employed in the manufacture of emetine or in handling powdered ipecac root not infrequently develop a cutaneous eruption as a result of the local action of the drug. A noticeable feature of this irritating action is the slowness of its onset. When injected hypodermatically the pain or soreness does not develop for several hours, sometimes not until the second day. When solutions of emetine hydrochloride, or even particles of the alkaloid itself, come into contact with the conjunctiva, pain is not felt for six or eight hours and severe inflammation does not set in previous to this time.

Conjunctival tests

In order to determine the relative irritating effects of cephaeline and some of its derivatives, solutions of these alkaloidal salts were instilled into the eyes of rabbits. It was determined that one-tenth per cent solutions were well suited for this purpose. The alkaloidal salts were dissolved in 0.85 per cent sodium chloride solutions and the right conjunctival sac was filled with the fluid to be tested. This was held in the sac for one minute and then allowed to flow out naturally. The left eye was treated in a similar manner with 0.85 per cent salt solution and was used as a control. The condition of the ocular and palpebral conjunctiva was observed from time to time over several days. The following protocol illustrates this method.

Thirty-two tests of this nature were made using cephaeline hydrochloride, emetine hydrochloride, cephaeline propyl ether phosphate, cephaeline iso-propyl ether hydrochloride and hydrobromide, cephaeline iso-amyl ether hydrochloride and hydrobromide, and cephaeline allyl ether hydrobromide. Of these substances cephaeline hydrochloride and emetine hydrochloride

TIME	RABBIT	AGENT	PALPEBRAL CONJUNCTIVA	OCULAR CON-JUNCTIVA
9.43	1	0.1 per cent ceph-aeline propyl eth-er phosphate	Normal appearance	Normal appearance
9.45	2	0.1 per cent emetine hydrochloride	Normal appearance	Normal appearance
After 3 hours	1		Injected	Injected
	2		Injected	Injected
After 7 hours	1		Injected	Injected
	2		Injection greater than rabbit 1	Injected
After 23 hours	1		Injection slight	Normal
	2		Marked injection and increased se-cretion. Edges of lids reddened. Much worse than rabbit 1	Injected
After 49 hours	1		Normal	Normal
	2		Still markedly in-jected, less than at 23 hours	Injected
After 97 hours	1		Normal	Normal
	2		Injection still pres-ent	Normal
After 121 hours	1		Normal	Normal
	2		Normal	Normal

were undoubtedly the most irritating to the conjunctiva. The irritation was intense and long lasting. Of the other sub-stances the cephaeline iso-amyl ether was the least irritating, although the difference among these was not very great.

These experiments were not carried further as at this time the fallacy of the results was discovered. On the assumption that cephaeline iso-amyl ether hydrochloride was considerably less irritating than emetine hydrochloride, $\frac{1}{2}$ grain of this substance in 1 cc. of sterile water was injected subcutaneously into the

deltoid region of one of the laboratory workers. This injection was followed by a similar one on each of the two succeeding days and on the fourth day a deep intramuscular injection of $\frac{1}{2}$ grain was given. The intramuscular injection gave rise to only a mild reaction. The injections given subcutaneously gave rise to considerable local reaction which consisted of a reddening of the skin and a soreness which developed during the first twenty-four hours. These effects increased during the following forty-eight hours at the end of which time there was also some induration. Objectively from seventy-two to ninety-six hours there was improvement though subjectively the condition was said to be worse. At the end of ninety-six hours the soreness decreased too. The reaction gradually decreased and was practically cleared up at the end of two weeks, although a small indurated subcutaneous nodule could still be palpated at the site of one of the injections.

Injections of $\frac{1}{2}$ grain cephaeline iso-amyl ether hydrochloride were made intramuscularly in a second individual. The left arm received an injection into the triceps on the first day and was only slightly tender on the second day, was swollen, tender and painful on the third day, and the inflammation was rapidly subsiding on the fourth day. On the second day, an injection was made into the right triceps. This showed only slight tenderness on the following day and another injection was made into the left arm, this time into the deltoid. This last injection gave only slight evidence of irritation. The right arm was not sore on the day following the injection nor on the second day, but on the morning of the third day the patient wakened with a very sore arm and felt ill. This being Sunday the patient was not seen until the fourth day when he presented a swollen, slightly reddened and very tender right arm. The swelling began at the point of injection just above the deltoid insertion and extended over the triceps and outer aspect of the arm to the elbow. There was no soreness or swelling above the point of injection. The patient could not raise the arm. The swelling gradually subsided and the pain decreased so that the arm was practically normal at the end of the tenth day following the injection.

Three other patients who received injections of this preparation for amebic dysentery complained of considerable soreness at the sites of the injections.

These clinical tests seemed to conclusively prove that the conjunctival tests for irritation as carried out were not a reliable measure of the irritation afforded by hypodermatic injections. While emetine hydrochloride often causes severe local reaction when injected, we have never seen it cause as severe reactions as were produced by the cephaeline iso-amyl ether hydrochloride. Further laboratory tests which will now be described have substantiated the clinical findings, or rather have disproved the findings based on the conjunctival tests.

Intramuscular tests

Solution of the phosphate of each alkaloid was compared with a like solution of emetine phosphate. The emetine phosphate was injected deep into the right lumbar muscles of a rabbit and the substance to be tested into the left. These solutions in different experiments were given in amounts of either 0.25 cc. or 0.5 cc. The amount of alkaloid injected varied from 2 mgm. to 32.4 mgm. The injection of 32.4 mgm. into each side was occasionally fatal. The usual dilution was 16.2 mgm. in 0.5 cc., this being the concentration most commonly employed in clinical practice. The rabbits were chloroformed on the fourth day following the injection and the subcutaneous and intramuscular reactions of the two sides were carefully compared macroscopically. In these experiments the phosphates of the following cephaeline ethers were tested: methyl, ethyl, propyl, iso-propyl, butyl, iso-butyl, tertiary butyl, iso-amyl, and allyl.

Without going into detail to describe the differences in the intensity of reaction, the comparative irritating effects may be indicated as follows:

DOSE	EMETINE PHOS-PHATE	
		Cephaeline phosphate
16.2 mgm. in 0.5 cc.....................	+++	+++
		Cephaeline ethyl ether phosphate
16.2 mgm. in 0.5 cc.....................	++	++
32.4 mgm. in 0.5 cc................... .	++	++ died during night
		Cephaeline propyl ether phosphate
32.4 mgm. in 0.5 cc.....................	++	+++
32.4 mgm. in 0.5 cc.....................	++	+++
16.2 mgm. in 0.5 cc.....................	++	+++
16.2 mgm. in 0.5 cc.....................	++	+++
16.2 mgm. in 0.25 cc.....................	+++	++
16.2 mgm. in 0.25 cc.....................	++	+++
		Cephaeline isopropyl ether phosphate
16.2 mgm. in 0.5 cc.....................	++	+
16.2 mgm. in 0.5 cc.....................	+	+
16.2 mgm. in 0.5 cc.....................	++	++
32.4 mgm. in 0.5 cc.....................	++	++ (died after 1 day)
		Cephaeline butyl ether phosphate
32.4 mgm. in 0.5 cc.....................	++	++
32.4 mgm. in 0.5 cc.....................	+++	++
16.2 mgm. in 0.5 cc.....................	+	+++
16.2 mgm. in 0.5 cc.....................	++	++
		Cephaeline iso-butyl ether phosphate
32.4 mgm. in 0.5 cc.....................	+++	++
16.2 mgm. in 0.5 cc.....................	+++	++
		Cephaeline tertiary butyl ether phosphate
32.4 mgm. in 0.5 cc.....................	++	+
16.2 mgm. in 0.5 cc.....................	+	++
		Cephaeline iso-amyl ether phosphate
32.4 mgm. in 0.5 cc.....................	++	+++
16.2 mgm. in 0.5 cc.....................	++	+++
16 2 mgm. in 0.5 cc.....................	+++	++++
16.2 mgm. in 0.5 cc.....................	++	+++
		Cephaeline allyl ether phosphate
16.2 mgm. in 0.5 cc.....................	+++	+++
16.2 mgm. in 0.25 cc.....................	+++	++
16.2 mgm. in 0.5 cc.....................	++	+++
16.2 mgm. in 0.5 cc.....................	++	++

Slight inflammation (+), severe inflammation (++), very severe inflammation (+++), necrosis (++++).

As may be seen the various derivatives were not tested against each other in the same rabbit, but each was tested against emetine phosphate, so that these results only in a general way indicate the relative irritating effect of the alkaloids tested. Suffice it to say that the cephaeline iso-amyl ether phosphate, which was least irritating in the conjunctiva tests, was the most irritating when given intramuscularly. Cephaeline and all of its derivatives tested by this method were quite irritating when given in the above dilutions and none of them possessed any decided advantage over emetine in this respect.

We have personally injected doses of $\frac{1}{2}$ grain cephaeline iso-amyl ether hydrochloride in 1 cc. in four patients and have had reports of four other cases in which this drug was used. In all cases severe pain and local inflammation was caused. We have made from three to six injections of $\frac{1}{2}$ grain cephaeline propyl ether hydrochloride or phosphate at daily intervals in twelve patients and have reports from similar injections in several others and in none of them was there more than slight local soreness at the point of injection. In three of these patients emetine hydrochloride was also injected for comparison and no difference could be noted in its local action and that of cephaeline propyl ether hydrochloride.

It may be that the apparently lessened irritating effect of these derivatives of cephaeline when tested on the rabbit's eye and their lowered toxicity when given subcutaneously to rats and guinea pigs is due to their being more difficultly absorbed from the tissues. Those compounds difficult of absorption would be washed out of the eye before seriously affecting the conjunctiva and when injected intramuscularly these compounds would be more irritating on account of their remaining longer in contact with the tissues. The further fact that their toxic dose bears an entirely different relation to emetine when given intravenously and when given subcutaneously would tend to confirm the above assumption.

In connection with the irritating action of the alkaloids of ipecac, we would like to record some incidental observations on the action of powdered ipecac root in producing "ipecac asthma."

In our laboratories there are seven men in whom typical bronchial asthmatic attacks occur whenever they come in contact with ipecac dust in the air.   On days when ipecac is being ground in the drug mills these men, although they may be one or two buildings distant from the grinding room, will suffer from asthma.   In some cases this attack is so severe as to temporarily incapacitate them.   In some the attacks are mild and pass off after a short time in the open air.   In others the attack may be mild at first and then become more severe at night after the patient has gone home and the effects may be noticed for as long as a week. Six of these cases have never had asthma at any other time or from any other cause.   On the other hand certain individuals who are subject to asthma and who frequently come in contact with powdered ipecac are not affected by it.   In four of the above cases adrenalin hydrochloride has been given hypodermatically and relieves the difficult breathing quite promptly.   That this ipecac asthma is not due to the alkaloids is evident, for no one handling either cephaeline or emetine has ever made such complaint.   Two of the men mentioned above are chemists and frequently come in contact with the alkaloids but are never affected by them.   Powdered ipecac root, from which the alkaloids have been removed, is still capable of producing these asthmatic attacks in susceptible persons, so that this would seem to be another instance of vegetable protein sensitization.

CONCLUSIONS

These experiments have demonstrated that in cats the emetic dose of emetine hydrochloride is approximately twice that of cephaeline hydrochloride, and that the higher homologues of this series decrease in emetic power very much in the same ratio as they do in toxicity, as reported in a previous paper (1).   Furthermore, it has been shown that the hydrochloride, hydrobromide and hydroiodide of emetine vary only slightly in their emetic power, but that the hydroiodide of cephaeline iso-amyl ether, due to its relative insolubility, is about one-half as emetic as the hydrobromide or hydrochloride of cephaeline iso-amyl ether and only one-sixth as emetic as emetine hydrochloride.

When tested on the conjunctiva of rabbits, emetine and cephaeline are the most irritant of this series and cephaeline iso-amyl ether is least irritating.

When injected intramuscularly in rabbits cephaeline iso-amyl ether is the most irritant while the difference between the other less irritant members of the series is not marked.

Cephaeline propyl ether phosphate gives no more than a slight local reaction when injected hypodermatically into persons, while cephaeline iso-amyl ether salts cause severe pain, soreness and local inflammation.

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A METHOD FOR THE STANDARDIZATION OF THYROID PREPARATIONS

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The treatment of conditions due to physiological inefficiency of the thyroid gland by the administration of preparations of the gland has given the thyroid a conspicuous place as a therapeutic agent. Its value was at first frequently doubted, as a result of conflicting reports of the results obtained by its administration, but as the structure and functions of the gland were investigated it became known that the physiological efficiency of the thyroid bore an intimate relationship to its iodine content and that much of the reported uncertainty of action was due to the use of products poor in iodine. The value of this gland as a therapeutic agent has been made more certain by standardizing thyroid preparations, on the basis of their iodine content.

It is known that the iodine content of the thyroid varies with the histological structure of the gland (1) and that the physiological activity of the gland, when administered, is dependent upon the iodine which it contains in combination with its colloid (1 and 2). Marine, in 1907, visited various manufacturing plants and found that hyperplastic, as well as normal glands, were being used in the commercial preparations, and he called attention to the necessity of determining the iodine content, leading to the adoption of the iodine standardization by all leading manufacturers and in the United States Pharmacopoeia (ninth revision).

IS THE IODINE CONTENT AN ENTIRELY RELIABLE INDEX TO THE THERAPEUTIC VALUE OF THYROID PREPARATIONS?

The iodine assay has served very well to improve the grade of physiologically-active preparations on the market and to eliminate many of the worthless inert products. But, since "inorganic iodine" does not serve the purpose in conditions where "thyroid iodine" is indicated, it is clear that the quantitative determination of iodine in a thyroid preparation can not be an entirely reliable index of its activity, for it is a simple matter to cause a hyperplastic, therapeutically worthless, specimen of thyroid to assay any amount of iodine, by simply adding iodine in any form.

The specific affinity of the thyroid for iodine is very well known and it has been shown that the hyperplastic gland is capable of absorbing large amounts of iodine, both in vivo and in vitro, and that when potassium iodide is injected into the circulation of an animal with hyperplastic thyroid glands, the iodine is absorbed by the gland practically as soon as it reaches the gland through the circulating blood (3 and 4).

If, a short while before being killed, a goiterous animal receives, in its food, a quantity of any salt of iodine, the thyroid will, of course, absorb it and on being assayed will show a good iodine content. Although, on assay, the iodine in such glands is present in sufficient quantities, it is not of value, when administered, in the sense of "thyroid iodine," as an activator of metabolism, as in the normal gland; for it has been shown that the iodine so absorbed by the thyroid requires some time (about twelve to twenty hours) before it is converted into the active "thyroid iodine" or the "iodine-containing hormone" of the thyroid (5).

In fact, the iodine of normal colloid glands is not entirely in the active form. Kendall (6), by alkaline hydrolysis, has obtained, from thyroid, three iodine-containing products, only one of which (product A) represents the essential activity of the gland. The writer, in collaboration with Dr. Marine (7) has recently confirmed that product A represents the active iodine

of the hydrolytic products of thyroid by feeding the products to tadpoles and further noted that product A, when obtained from hyperplastic (iodine-free) thyroid, was artificially iodized it did not produce the specific effect upon tadpoles although it contained a high percentage of iodine.

From the foregoing, it is evident that standardization of thyroid by determination of its iodine-content is not an entirely reliable indication of its therapeutic value and that a method of physiological assay as a supplement or substitute for the present method of iodine assay is very desirable. Such a method, to be useful, must of course be simple and practical.

Hunt (8 and 9) described a method in which mice were used as the test objects, and found that feeding of thyroid was capable of increasing their resistance to certain poisons (acetonitril). Having had no personal experience with this method, I am not in a position to offer comment on it, but since the author found that many other conditions, such as a large variety of diets, feeding of most of the glands concerned with reproduction, state of nutrition of the animal, etc., were capable of eliciting effects similar to that produced by thyroid feeding, although not as marked, it may be concluded that this reaction is not specific and is necessarily variable.

It must be considered that since the thyroid is capable of producing very marked physiological changes in an animal, it must be important to have as test objects animals whose thyroid glands are constant as regards their structure and physiological state of efficiency. Indeed, Hunt indicates that the thyroid and other iodine compounds, which he tested by this method, may act through their influence upon the animals thyroid glands. Since the thyroid gland, of all organs, is the one most subject to variations in structure and function, it seems likely that this method must yield results varying considerably with the condition of the thyroids of the test animals. However, the method has apparently given good results in the hands of its author, and his conclusions are similar to those obtained by the method employed by me.

For example, it was found by Hunt and Seidell (10) that iodine

must be in proper combination in the thyroid to be physiologically effective and that the therapeutic efficiency of the thyroid is in direct proportion to the amount of iodine in such combination.

The remarkable specific effect on tadpoles of thyroid feeding, described by Gudernatsch (11) is extremely delicate and can be easily observed.

Lenhart (12) showed that desiccated thyroid when fed to tadpoles causes effects on their growth and differentiation in proportion to the quantity fed and the amount of iodine present, and indeed, he suggests that this sensitive reaction might serve as a biological test for the activity of the thyroid, superior even to chemical methods.

As mentioned earlier in this paper, we further found with the tadpole test that not only is the effect in proportion to the amount fed and the amount of iodine present in the gland, but that only the iodine in proper combination is capable of eliciting the specific effect. This result was the basis for the elaboration of the method of standardization described in this paper.

Seven specimens of thyroid were obtained in retail prescription pharmacies, and were represented by the following products:[1] Parke, Davis and Company (tablets and capsules), Armour and Company (powder), Burroughs-Wellcome Company (tablets), Mulford and Company (tablets), and The Phospho-Albumen Company[2] (powder). A specimen of desiccated cattle thyroid, extracted with gasoline and ether, which was prepared in this laboratory from glands carefully selected as normal (from gross appearance) by Dr. Marine and myself, and having an iodine content of 0.215 per cent (assayed by Dr. Marine), was used as a standard. This preparation had previously been used for other investigations in this laboratory and its products of hydrolysis had been studied. This increases its reliability as

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[1] The firm names are given for the purpose of identification. It must be remembered that the preparations were bought in the retail market and are therefore of uncertain age, and also that this test has not been in use by commercial firms.

[2] The druggist had this preparation in stock a very long time, being uncertain of its age.

a standard. The product A of this specimen of thyroid contained 1.48 per cent of iodine, product B, 0.12 per cent, and the residue 0.028 per cent. The amount of product A obtained was about one-eighth of the weight of the total amount of thyroid hydrolyzed. This indicates that in this specimen there is at least about 15 per cent of inactive iodine, since only product A represents the activity of the preparation. Indeed, product A showed an activity about twelve times as great as the thyroid from which it was obtained, although its iodine content was only seven times as great, again indicating that a substantial proportion of the thyroid iodine was inactive.

DETAILS OF METHOD

A stock of tadpoles (larvae of Rana pipiens) was brought to the laboratory on May 26, 1917, and placed in large basins of tap water on tables in an airy and well lighted room. The stock tadpoles were fed with fresh liver on alternate days and the water changed daily, or on hot days twice daily. From the stock were selected a number of tadpoles of uniform size and these were placed in small enamelware dishes of about 200 cc. capacity, each dish being about three-fourths full of tap water and containing five tadpoles. The same plan of feeding was carried out as was followed by Lenhart. Each series was studied in duplicate. The water in these dishes was changed twice daily, the tadpoles were fed fresh liver every other day and the thyroid (in a moderately coarse powder) was fed on the alternate days. It was found convenient to feed the liver about two or three hours before the second change of water, so that it would not remain in the dishes over night, thus preventing putrefaction in hot weather. The thyroid was fed after the second change of water and remained in the dishes until the next morning, when the water was changed and the dishes rinsed thoroughly. Along with each series were placed three dishes, each containing five tadpoles, these serving as controls. The controls were given only liver on alternating days and their water was changed twice daily. The dishes were placed in duplicates upon long tables in

TABLE 1

PREPARATION	IODINE CONTENT (per cent)	DOSE FED (mgm.)	FIRST EFFECT IN (days)	DOSES GIVEN	AMOUNT REQUIRED (mgm.)	REMARKS	DEFINITE EFFECT IN (days)	DOSES GIVEN	AMOUNT REQUIRED (mgm.)	REMARKS	PHYSIOLOGICAL VALUE COMPARED TO STANDARD
A	0.215*	30	2	1	30	Angulation of head; slight loss of weight	5	3	90	Most of tails absorbed, hind legs appearing; marked emaciation	100
		10	3	2	20	Angulation of head; slight loss of weight	7	4	40	Hind legs appearing; marked emaciation	
		5	6	3	15	Slight angulation of head; slight loss of weight	12	6	30	Marked loss of weight; heads angular	
		2	10	5	10	Slight angulation of head; slight loss of weight	14	7	14	Emaciation	
B	0.2†	30	2	1	30	Marked angulation of head; marked loss of weight	4	2	60	Marked emaciation and differentiation, hind and forelegs; most of tails absorbed	100 + 20
		10	2	1	10	Marked angulation of head; marked loss of weight	6	3	30	Marked emaciation and differentiation, hind and forelegs; most of tails absorbed	
		5	4	2	10	Angulation of head; loss of weight	7	4	20	Marked emaciation and differentiation; most have hind legs	
		2	9	4	8	Angulation of head; slight loss of weight	11	6	12	Marked emaciation; four have hind legs	
C	0.2†	30	2	1	30	Marked angulation of head; marked loss of weight	4	2	60	Marked emaciation, hind and forelegs; most of tails absorbed	100 + 10 to 20
		10	2	1	10	Marked angulation of head; loss of weight	6	3	30	Marked emaciation, hind and forelegs; most of tails absorbed	
		5	5	3	15	Angulation of head; loss of weight	8	4	20	Marked emaciation; eight have hind legs	
		2	9	4	8	Slight angulation of head	11	6	12	Marked emaciation;	
D	0.2†	30	2	1	30	Angulation of head; loss of weight	4	2	60	Most of tails absorbed, hind legs; some emaciation	100+

		10	3	2	20		6	3	30		
	·	5	5	3	15	Angulation of head; loss of weight	10	3	25	Most of tails absorbed, hind legs; some emaciation	
		2	10	5	10	Angulation of head; loss of weight	14	7	14	Six have hind legs	
E	0.2†	30	2	1	30	Slight angulation of head; slight loss of weight	5	3	90	Marked emaciation	100
		10	3	2	20	Angulation of head; loss of weight	6	3	30	Most of tails absorbed, hind legs; marked loss in weight	
		5	6	3	15	Angulation of head; loss of weight	11	6	30	Hind leg buds; marked loss in weight	
		2	11	5	10	Slight angulation of head; slight loss of weight	14	7	14	Marked emaciation; two have hind legs	
						Slight angulation of head				Marked emaciation	
F	0.05‡ in fresh gland	30	3	2	60	Marked angulation of head; marked loss of weight	5	3	90	Marked emaciation, tails absorbing; some have hind legs	50 to 60
		10	5	3	30	Angulation of head; loss of weight	8	4	40	Marked emaciation and differentiation	
		5	9	5	25	Angulation of head; slight loss of weight	14	7	35	Marked emaciation, hind legs budding	
		2	13	7	14	Angulation of head; slight loss of weight	14	7	14	Increased emaciation	
G	0.136*	30	5	3	90	Angulation; loss of weight	9	5	150	Marked loss of weight; few have hind legs	40 to 50
		10	7	4	40	Slight angulation; loss of weight	12	6	60	Marked loss of weight	
		5	13	7	35	Slight loss of weight	14	7	35	Increased emaciation	
		2	14	7	14	Practically no action—growth	14	7	14	Growth	
H	0.17*	30	11	6	180	Slight angulation; loss of weight	14	7	210	Increased loss of weight; more angular	10 to 20
		10	13	7	70	Slight loss of weight	14	7	70	Increased loss of weight	
		5	14	7	35	Practically no action—growth	14	7	35	Growth	
		2	14	7	14	No action—growth	14	7	14	Growth	
Controls						Progressive growth					

* I am indebted to Dr. Marine for these iodine determinations.

† Specimen labeled "containing not less than 0.2 per cent of iodine" (manufacturer's assay).

‡ Not less than 0.05 per cent in the fresh gland would correspond to about 0.2 per cent in the desiccated gland.

a large room, which was well ventilated and free from chemical
fumes.   The light in the room was good.

A preliminary series was observed, to form a general idea of
the approximate comparative values of the different prepara-
tions.   In this set, which was started on May 29, 1917, the tad-
poles were fed with 60, 30, 10, 5 mgm. doses of each prepara-
tion.   Having ascertained the relative effects of the various
products, another series was started, on June 6, 1917, the doses
being reduced to 30, 10, 5, 2 mgm., it being found that 60 mgm.
was too high a dose to give comparative results, as the action
produced was, in most cases, too rapid.   A tabulation of the
notes of this series, in condensed form, is given in table 1.

A third series, with doses of 20, 10, 5 mgm. was started on
June 20, 1917, with older tadpoles and gave results closely corre-
sponding to the second series.   It was found that the best re-
sults were obtained with tadpoles somewhat older (two to four
weeks) than those employed in the first series (seven to ten days).
The effect was most easily demonstrated in tadpoles whose bodies
were about 6 to 8 mm. in length.

The estimation of the physiological activity is easily done
by observing the dose and time required for a certain effect on
differentiation and growth to become manifest.   It is a simple
matter to determine the amount of a given preparation of thy-
roid necessary to produce a like effect in the same time.   Phys-
iological methods of assay cannot, at best, be as accurate as
chemical methods.   However, a difference of more than 10 per
cent of the active iodine could be easily detected by this method
and it is certainly not important to determine the physiological
value of a therapeutic agent of this type to a closer degree than
about 10 per cent.   The relative values of the different prepara-
tions are expressed in the table with reference to the cattle
thyroid preparation taken as 100.

It will be seen from the results tabulated that the effects pro-
duced by specimen G is roughly in proportion to its iodine con-
tent and also that specimen F, which contained the same amount
of iodine as the active specimens, produced an effect equal to
only about one-half the effect of the standard, while specimen H

showed a very low activity in comparison with its iodine content. This again emphasizes the fact that the value of thyroid is in proportion, not to its total iodine, but to the iodine in combination.

The possible objection to this method may be made that tadpoles are not available at all times of the year.   Since the growth and development of the stock tadpoles can be retarded for a relatively long period, by keeping them in a cold place, it is possible to utilize nearly all of the summer months for this kind of work and it is probable also that by raising tadpoles and frogs on a large scale, it might be possible to regulate temperature and other conditions so that tadpoles could be available during a greater part of a year.   At any rate, stock of thyroid accumulated in the winter would be available for assay during the proper period, since it would not change if kept with reasonable precautions.

SUMMARY

A method is described in which the specific action of thyroid upon tadpoles is used to assay the physiological value of commercial thyroid preparations.

Of seven products purchased in retail drugstores, two indicated about 20 per cent more activity than a standard preparation, two were equal in value to the standard, one somewhat more than 50 per cent of the standard, one less than 50 per cent, and one was a practically worthless preparation—being only about 10 to 20 per cent of the value of the standard.

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THE INFLUENCE OF OXYGEN UPON INFLAMMATORY
REACTIONS

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Experiments by Amberg and Knox (1), Amberg (2), and
Arkin (3) have shown that various inflammatory reactions are
markedly inhibited by intravenous injections of the sodium
salts of o-iodoso- and o-iodoxy-benzoic acids. In these experi-
ments the agents producing the inflammation had been admin-
istered intracutaneously. Bruce (4), Chiari and Januschke (5)
reported an inhibiting effect of various substances on the in-
flammatory edema of the conjunctiva of the rabbit produced
by the instillation of mustard oil. They took the precaution
of lifting the lower lid well away from the eye ball, dropping the
oil in the sac thus formed. In this manner it is possible to
obtain rather uniform results. In the following series of experi-
ments our substances were injected intravenously while the mus-
tard oil was instilled in the conjunctival sac. The instillation of
mustard oil in the eye of the normal rabbit is followed immedi-
ately by a spasm of the lids and weeping. Within a short time
the spasm relaxes, the eye opens and the conjuctiva is seen
deeply congested with the pupil contracted. Edema of the
palpebral and ocular conjunctiva develops, reaching its maxi-
mum in about an hour. The lids stand out well and there is
some eversion of edematous tissue. The cornea can no longer
be exposed entirely. Keeping step with the development of the
edema the conjuctiva blanches. The next day the edema has
usually diminished very much, while the hyperemia has reestab-
lished itself. The lids may be found glued together by a

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creamy exudate filling the conjunctival sac. The cornea is opaque. The effect of the intravenous injections of the drug mentioned will be illustrated by a few examples.[1]

Rabbit 7. March 4, 1914. 1300 grams

2.45 p.m.   10 cc. $\frac{N}{20}$ sodium iodoxybenzoate.
2.50 p.m.   1 drop mustard oil in left conjunctival sac. Spasm of
               lids.
2.55 p.m.   Eyes open. Hyperemia of conjunctiva.
3.00 p.m.   Same.
3.30 p.m.   Eye appears normal.
4.00 p.m.   Same.
7.45 p.m.   Same.
   March 5, 1914, 10 a.m. A little purulent exudate. Otherwise nothing to be seen.

Rabbit 9. March 5, 1914. 1170 grams

3.32 p.m.   10 cc. $\frac{N}{20}$ sodium iodoxybenzoate.
3.34 p.m.   1 drop of mustard oil in left conjunctival sac.   Spasm of lids.
3.50 p.m.   Eye open, no edema, slight redness.
4.55 p.m.   Same.
   March 6, 1914, 10 a.m. Lids glued together, creamy pus in sac. Corneal opacity.

Rabbit 99. June 7, 1914. 920 grams

3.20 p.m.   5 cc. $\frac{N}{20}$ sodium iodoxybenzoate immediately followed by
               1 drop of mustard oil in right conjunctival sac.
3.50 p.m.   Slight redness, weeping, no edema.
4.20 p.m.   Redness, slight edema.
5.20 p.m.   Marked edema.
   June 8, 1914, 10 a.m. Slight opacity of cornea, edema, redness.

Rabbit 19. March 10, 1914. 2075 grams

3.00 p.m.   10 cc. $\frac{N}{20}$ sodium iodosobenzoate, then mustard oil in
               right conjunctival sac.
3.05 p.m.   Eye open, weeping, hyperemia.
3.15 p.m.   Eye closed, weeping, redness, no edema.
3.30 p.m.   Eye open, no edema, slight hyperemia.

[1] In a part of the work we were assisted by Dr. P. S. Chancellor.

4.00 p.m.   Same.
4.30 p.m.   Eye apparently normal.
5.30 p.m.   Same.
7.35 p.m.   Slightly pink, otherwise normal.
   March 11, 1914, a.m.   Eye apparently normal.

It may be remarked that all our experiments were accompanied by controls. The intravenous injection of sodium iod-benzoate had no inhibiting effect, and the protocol of one of these experiments may be taken as an illustration of the course of the conjunctival mustard oil reaction in an untreated animal.

Rabbit 28. March 13, 1914. 975 grams

3.40 p.m.   10 cc. $\frac{N}{20}$ sodium iod-benzoate; immediately afterwards 1 drop of mustard oil in left conjunctival sac.
3.45 p.m.   Eye closed, weeping, some redness.
4.00 p.m.   Considerable edema, slight redness and weeping.
4.10 p.m.   Marked edema.
4.20 p.m.   Edema progressed.
4.30 p.m.   Very pronounced edema. Difficult to expose more than about half of the cornea.
5.00 p.m.   Edema about same.
9.25 p.m.   Edema less, white secretion, lids glued together.
   March 14, 1914. Lids glued together, creamy exudate, redness, edema much diminished.

Some control experiments were also made with 5 per cent NaCl, of which 10 cc. were injected intravenously in two rabbits of 1410 and 1210 grams weight without any effect on the intensity of the reaction. Furthermore, control experiments with sodium salicylate seemed advisable, for Jahn (7) detected traces of salicylic acid in the urine of rabbits after intravenous injections of sodium iodoso benzoate. Januschke (6) gave rabbits of 1400 to 1600 grams, in half hourly intervals, three doses of 0.5 gram sodium salicylate subcutaneously. At the time of the last injection mustard oil was instilled in the eye. A very marked inhibition of the inflammation was noted. We injected three rabbits of 1330 to 1520 grams each with 10 cc. $\frac{N}{10}$ sodium salicylate intravenously, without any definite inhibiting effect on the conjunctival mustard oil reaction.

When the edema is completely or nearly completely inhibited by sodium iodoxybenzoate, the weeping and the hyperemia occuring immediately after the instillation of the mustard oil may disappear within about half an hour. Sometimes, it is hardly possible to determine which eye has been used. Rather frequently the eyes of control animals with a rapidly progressing edema, show less congestion than the eye of animals whose edema is markedly inhibited. However, in spite of a nearly complete inhibition of the edema sometimes the cornea was found cloudy the next day and there was considerable creamy exudate. In some cases as in many of the controls the opacity of the cornea was very pronounced, and persisted, while in others the eye recovered entirely. The cloudiness of the cornea may begin three to four hours after the instillation of the oil.

We may summarize our results by saying that the intravenous injection of sodium iodoso- or iodoxy-benzoate inhibits the inflammatory edema produced by mustard oil in the conjuctiva of the rabbit, while the sodium iod-benzoate has no such effect. It appeared that the degree of inhibition is roughly proportional to the dosage. Furthermore, the sodium iodosobenzoate seemed to be more effective than the sodium iodoxybenzoate.

Some animals having received intravenous injections of sodium iodoso- and iodoxy-benzoate, with consequent marked inhibition of their conjunctival mustard oil reactions, were sacrificed one hour after the injection. The viscera did not show any macroscopical or microscopical lesions.

It had been noted that the administration of our substances was followed by some diarrhoea. Sometimes this diarrhoea became very profuse. When this happened the experiments were excluded from consideration. Nevertheless, it became desirable to determine definitely whether any importance attached to the diarrhoea as a factor in the observed inhibition of the mustard oil reaction. It should be pointed out that quite pronounced diarrhoea had been seen occasionally in control animals which gave very good inflammatory reactions. Two possibilities had to be considered; the first was the actual loss of

fluid from the organism, the second. a possible effusion of fluid in the small intestines without a manifest diarrhoea. Some few attempts at obtaining a pronounced but controlable diarrhoea in rabbits by means of a combined treatment with saline cathartics and pilocarpin or physostigmin, did not yield reliable results. Our question was answered satisfactorily, however, in the following manner: A female rabbit, after expressing the contents of the bladder, weighed 1800 grams just before the experiment was started. It received a continuous injection of a 0.4 per cent solution of iodoxybenzoic acid as sodium salt in the marginal ear vein by means of a Woodyatt (8) motor syringe. Twenty-three minutes after the beginning of the injection, when the rabbit had received 12 cc. of the solution, 2 drops of mustard oil were instilled in the conjunctival sac, without interrupting the injection, which was continued for forty-seven minutes longer. During this time 26 cc. were injected, giving a total of 38 cc. At the end of the injection the rabbit passed spontaneously a small well formed stool. A small amount of urine and some more fecal balls were expressed. The rabbit weighed 1830 grams. From then on until it was killed two and one-half hours after the instillation of the mustard oil no more stool or urine was passed. In the beginning of the experiment there was some secretion from eyes and mouth, the latter keeping up throughout the injection, but as can be seen from the weight of the animal this salivation was very moderate indeed. The inhibition of the mustard oil reaction, while not complete, was very marked. At the same time the mustard oil was applied to this animal a control rabbit was treated alike and responded in the usual manner with a fully developed edema, in spite of the fact that thirty minutes after the instillation of the mustard oil it passed some rather fluid stools and continued to do so for about 20 minutes. It was also killed two and one-half hours after the application of the mustard oil.

The rectum of the injected animal contained well formed dry fecal matter. The content of the large intestine seemed to be of about the usual consistency and the small intestines did not appear unusually filled. The rectum of the control animals con-

tained semisolid unformed fecal matter and the contents of the large intestine were decidedly more moist. The experiment was repeated with the same result as far as the effect of the intravenous injection of the sodium iodoxy benzoate was concerned the animal being killed seventy-five minutes after the instillation of the mustard oil. The fortunate accident of a control with a marked diarrhoea did not reoccur. It may be mentioned that several times rabbits having received several drops of mustard oil had a transitory slight diarrhoea, that is, they passed rather more fecal balls, decidedly moister and less well formed than usual.

We have here, then, a very marked inhibition of the mustard oil reaction without a trace of a diarrhoea while a control with a rather profuse diarrhoea did not show any inhibition. Neither did we have any indication of an effusion of fluid anywhere in the organism, a conceivable factor in the inhibiting action. Furthermore, neither in these experiments nor in those mentioned above where a post mortem examination was made, did we have any indication of any irritation caused by our injected substances, a point to which we will have to refer further on.

It now became our aim to study the effect of other organic substances containing chemically active oxygen in other combinations, and furthermore some inorganic salts with active oxygen. The selection of substances suitable for our purposes is very limited (see Grove and Loevenhart (9)).

In previous experiments (2) with the disodium salt of diphthalic peroxide (see Bayer and Villiger (10)).

$$C_6H_4 \diagup \overset{\displaystyle COO \text{------} OOC}{\diagdown COOH \ HOOC} \diagdown C_6H_4$$
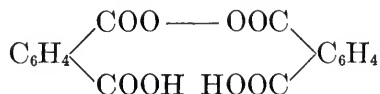
no inhibiting effect could be seen. The solution then used was $\frac{N}{20}$ but contained only 53 per cent of the theoretical amount of active oxygen. The solution now used was $\frac{N}{10}$ and contained 95.6 per cent of the theoretically required active oxygen. The solution prepared with the theoretical amount of NaOH was still very slightly acid. The intravenous injections were made

immediately after the preparation of the solution and the utmost care had to be observed in making the injections. The mustard oil was given intracutaneously, 0.1 cc. of a 1 per cent solution in olive oil. A rabbit of 2200 grams and another of 2060 grams received each 10 cc. and a third rabbit of 1380 grams received 6.5 cc. of the $\frac{N}{10}$ sodium perphthalate. The inflammatory reactions were measured in mm. as in previously reported experiments (2) and seven and one-half hours after the injections,[2] with the following average results:

	THREE HOURS	SEVEN AND ONE-HALF HOURS
	mm.	mm.
Controls..	32	41
Experiment.....................................	25	35

After three hours the cutaneous swelling of the treated animals was rather diffuse, ill defined and not pronounced and we had at this time an inhibition more marked indeed than indicated by the measurements. This inhibition became less and less pronounced as the time advanced. In another series we had only one successful experiment, several other animals dying within a few minutes after the intravenous injections. In this, a rabbit of 1080 grams received 6 cc. of a $\frac{N}{10}$ solution, and mustard oil in the eye; this animal had a marked inhibition of the inflammatory process without showing any toxic symptoms. The dry preparation from which the solution was made contained 5.35 per cent active oxygen, instead of 4.85 per cent, as required by theory. The solution required decidedly more than the theoretical amount of NaOH. The final solution contained about 90 per cent of the theoretical amount of active oxygen and may have been a mixture of $C_6H_4\begin{cases} COO\text{------}OOC \\ COONa\ NaOOC \end{cases}C_6H_4$

[2] The figures given for the measurements of the intracutaneous reactions were obtained by measuring two diameters in millimeters and taking the mean. Then the average of the reactions in a series of animals was struck. The figures therefore represent the average diameter of the reactions in millimeters.

and $C_6H_4\begin{smallmatrix}COOONa\\COONa\end{smallmatrix}$ and sodium phthalate. Control experiments with $\frac{N}{10}$ sodium phthalate were negative. The sodium salt of diphthalic peroxide exercises undoubtedly an inhibiting influence on the mustard oil inflammation.

Solutions of sodium iodate and sodium periodate were prepared by neutralizing the corresponding acids with NaOH. The concentrations used for intravenous injection was $\frac{N}{20}$. In one set of experiments, 0.1 cc. of a 2 per cent mustard oil solution was injected intracutaneously. Four animals from 2100 to 2340 grams received each 6.6 cc. sodium iodate, four animals from 2000 to 2400 grams 5 cc. sodium periodate four animals received 100 cc. O_2 gas intraperitoneally and four served as controls. The average results were as follows.

	TWO HOURS	FOUR HOURS	SIX HOURS
	mm.	*mm.*	*mm.*
Controls..............................	30	39	46
Sodium iodate..........................	24	33	41
Sodium periodate.......................	28	31	38
Oxygen gas.............................	30	39	44

In these experiments the iodates exercised undoubtedly an inhibiting effect, showing itself, as in the experiments with diphthalic peroxide, less in the area of the swelling than in its height. The average size of the reactions of the animals treated with sodium periodate two hours after the injection exceeded those of the animals treated with sodium iodate. Nevertheless the reactions of the periodate animals were decidedly the least intense ones throughout. The fact that the differences in the intensity of the reactions is not shown as well in their area as in those experiments reported in an earlier paper (2) may be due to the greater dilution of the mustard oil in these experiments.

Sets of two animals each served for experiments with instillation of the mustard oil in the eye and intravenous injections of $\frac{N}{40}$ solutions of sodium iodate and sodium periodate, and $\frac{N}{10}$

sodium iodide.   Two animals of 1304 grams received each 10 cc. sodium iodate solution, two of 1140 and 1300 grams received each 10 cc. sodium periodate solution, two of 1120 grams each 10 cc. NaI while two served as controls.   There was no difference between the reactions of the controls and those of the sodium iodide animals, but a marked inhibition was caused by the iodates with a decidedly stronger effect of the periodate.

It is to be noted that injections of 2 to 5 cc. $\frac{N}{20}$ sodium periodate in the femoral vein of etherized cats of 2 kgm. weight did not have any very marked effect on respiration or blood pressure.   On injection of a fatal dose the respiration becomes gradually slower and shallower until it ceases.   There is no effect on the respiration comparable to that of sodium iodoso and iodoxy benzoate (Grove and Loevenhart (9)).

Trials with sodium perborate were given up.   The injection is mechanically very difficult and the animals die very readily. Of a series of experiments only one animal of 625 grams survived 10 cc. of a 0.89 per cent solution.   There was some doubtful inhibition of the eye reaction.

Ammonium persulfate, of which a $\frac{N}{20}$ neutralized solution in 10 cc. doses was injected in three rabbits of about 1000 grams, did not produce a noteworthy, if any, inhibition of the conjunctival mustard oil reaction.   A peculiar result was obtained with a commercial solution of sodium hypochlorite.   The alkalinity of this solution was equal to $\frac{N}{10}$ and 1 cc. required 10.58 cc. $\frac{N}{10}$ thiosulfate solution.   One animal of 1590 and another of 2290 grams received each 10 cc. of a 1:5 freshly prepared solution intravenously.   A third animal of 1400 grams received 10 cc. of a 1:10 solution.   In all three animals mustard oil was instilled in the eye.   There was perhaps some lag in the development of the edema.   The hyperemia was very intense and there was a bloody exudation.   The first two animals survived and the next day showed less traces of their hemorrhages than was to be expected, indeed it is questionable if anything unusual would have been noted if it had not been for the peculiar character of the reactions the day before.   From both of these animals some bloody urine was obtained.   The third animal whose hemor-

rhagic edema had become pronounced one and one-half hours after the application of the mustard oil, was found dead in its cage the next morning. The eye lids still presented a marked edema suffused with blood. A part of the lids and also the eye ball were destroyed and bloody fluid filled the socket. Coagulated blood was in the cage and the urine receptacle contained very bloody fluid. The animal seemed to have bled to death from a ruptured vessel of the eye. The point we wish to emphasize in this connection is this: that the character of the inflammation may be altered by the introduction of certain substances into the circulation. In this case it is a substance having evidently an injurious effect on the vessels, as may be inferred from the appearance of bloody urine. In addition it should be noted that animals dying from the effect of the hypochlorite solution present hemorrhages as the most striking feature.

For several reasons we tried to administer substances by the intraperitoneal route, the effect of which we wished to study on the mustard oil inflammation. It was thought particularly desirable to study the effect of benzoyl peroxide, which had proved of value clinically as pointed out by Loevenhart (11). Benzoyl peroxide is very slightly soluble in non-irritating solvents. The intravenous injections of sodium iodoso- and iodoxy-benzoates are time consuming and not always very easy, and these compounds can not be given subcutaneously, because they produce severe irritation. Iodoxy-benzoic acid was given in solution as the sodium salt; iodoso-benzoic acid was given in suspension in saline, its solution as sodium salt having proved very irritating in the experiments of Grove and Loevenhart. Benzoyl-peroxide was also given in suspension through a small incision in the abdominal wall. The dosage of the iodoxy- and the iodoso-benzoic acid was the same as in the previous experiments, and the inhibiting effect was also apparently about proportional to the dose. The mustard oil was instilled in the conjunctival sac. As usual, sodium iod-benzoate had no inhibiting effect. Benzoyl-peroxide was given in large doses, 0.75 to 1 gram each, to two animals of 1500 to 2000 grams. One animal of each set showed a very pronounced inhibition, the others showed

about the same grade of edema as the controls which received 7.5 and 10 cc. of a 10 per cent solution of sodium benzoate intraperitoneally. Two animals of about 1600 grams received each 10 cc. of a 10 per cent solution of sodium chloride intraperitoneally without any effect on the reaction.

In order to test the possible effect of the introduction of foreign substances in the peritoneal cavity on the mustard oil reaction of the eye, a number of animals received Kaolin (Merck) in 2.5 and 10 per cent suspensions. It was found that as little as 0.1 gram Kaolin given to an animal of about 900 grams may produce a marked inhibition, but not always. As the result of fourteen experiments on animals weighing 800 to 1900 grams, killed one and one-half to twelve hours after the injection, we may state that the degree of inhibition is roughly parallel to the intensity of the peritoneal lesions produced by the injections. A marked inhibition was also noted on intraperitoneal injections of 10 cc. of a 5 per cent suspension of animal charcoal (Blutkohle, Kahlbaum) in three animals. It may be mentioned that the intracutaneous injection of small amounts of Kaolin in man is very painful and leads to a strong inflammatory reaction.

One of two animals received 1 cc. of a $\frac{N}{20}$ solution of sodium iodoxybenzoate per 100 grams bodyweight intraperitoneally, another a like dose of iodoso-benzoic acid in suspension. Both showed good inhibition of their mustard oil reaction, which in the latter case was nearly complete up to the time the animals were killed, that is, one hour after the treatment. The first animal showed only doubtful signs of peritoneal irritation, the other marked signs such as great excess of free fluid and numerous haemorrhages in the omentum. Three rabbits received sodium iodoxybenzoate, three iodoso-benzoic acid in suspension. All animals showed marked inhibitions. They were killed after three hours, when those treated with iodoso-benzoic acid had bloody fluid in the peritoneal cavity and fine haemorrhages on the serosa of the abdominal wall as well as of the large and small intestines. The animals treated with sodium iodoxybenzoate solution had no excess fluid and the pin-point haemorrhages were much less numerous. One of the animals having received

10 cc. 10 per cent NaCl was sacrificed after thirty-five min-
utes, when the edema of the lids was already very marked.
There was a considerable excess of perfectly clear fluid in the
peritoneal cavity while the serosa of the abdominal wall was
somewhat injected.

The results of experiments where substances are adminis-
tered intraperitoneally must be interpreted with great caution.
While it is very doubtful whether in the case of sodium iodoxy-
benzoate, peritoneal irritation enters into consideration with
regard to its inhibiting action, we will only very briefly refer
to a series of experiments undertaken to see whether the sodium
iodoxy-benzoate can exercise any influence when the edema is
once under way.   The administration ten minutes after the ap-
plication of the mustard oil, when a slight degree of edema
exists, causes a pronounced inhibition.   When given an hour
after the instillation of the mustard oil, that is at a time when the
edema is rather fully developed, no effect whatever was noted
in some cases, in others there may have been a slightly beneficial
effect, but in no case was this very striking.

In order to obtain some further information concerning the
influence of irritation elsewhere on the cause of the mustard oil
reaction of the eye, a few experiments were made in which
rabbits received mustard oil intraperitoneally or subcutaneously.
When 0.1 cc. of a 1 : 20 dilution of mustard oil was injected
intraperitoneally in two rabbits of about 1200 grams no definite
inhibition of the conjunctival inflammation was noted, with 0.3
cc. one animal of 1350 grams did not show any definite inhibi-
tion, the other of 1250 grams did.   Four hours after the injection
the animals were killed.   Those without any definite inhibition
had hardly any excess fluid in the peritoneal cavity and only a
few pin point haemorrhages on the serosa of the large intestine,
the one with marked inhibition had more fluid, a small area of
intense injection of the peritoneum at the site of the injection
and in several places the peritoneal covering of the large intes-
tine was studded with small haemorrhages.   Another animal of
1350 grams had received 0.5 cc.   No definite edema developed
before the lapse of one and one-half hours, then the edema be-

came well marked four hours after the injection when the animal was killed. The animal had been ill; it was restless, had a very rapid respiration, and was somewhat cyanotic. These symptoms subsided but gradually. At the time it was killed it appeared quite normal. The peritoneal cavity was filled with clear fluid containing some flakes. A gelatinous coagulum was lifted out of the fluid. The serosa of the abdominal wall as well as that of the small intestine was congested, but nowhere intensely. The serosa of the large intestine was the seat of very fine haemorrhages which were rather numerous in places. We have here a development of quite a fair degree of conjunctival edema in spite of the very marked effusion elsewhere. Two animals of 1275 and 1475 grams received each 0.5 cc. 1:10 mustard oil under the skin of the back. Up to the time the animals were killed, that is for three hours, the development of the conjunctival edema of the first animal was very markedly inhibited, that of the second animal not much. The lesions produced by the subcutaneous injections did not differ appreciably in intensity.

The deduction we can draw from these experiments is that a certain degree of inhibition of the mustard oil inflammation of the conjunctiva may be brought about by irritation in other parts of the body, be it in the peritoneal cavity or elsewhere, as under the skin. These experiments call to mind the result of R. Winternitz (12) who studied the inhibiting action of etheral oils on inflammation. He describes experiments with the instillation of a weak solution of bee poison in the conjunctival sac of the rabbit. An animal treated per os with santal oil did not show any reaction while the untreated one showed some conjunctival hyperemia. Furthermore, if we are not mistaken, these experiments are the first to give some support to the clinical conception of the value of counter irritation in inflammation.

In the following experiments we tried to study the effect of diminished tissue oxidations on the mustard oil inflammation.

In a series of experiments rabbits were exposed to a reduced partial pressure of oxygen keeping the atmospheric pressure unchanged. The apparatus of Kolls and Loevenhart (13) was

used.   The mustard oil was given intracutaneously, 0.1 cc. of a
1 per cent solution in olive oil, prepared freshly for each series.
Four rabbits with four controls served for the first experiment.
·The four rabbits were put in the apparatus and the oxygen
content gradually reduced.   From the time the oxygen had
dropped to 12.4 per cent until the mustard oil was injected,
sixteen hours elapsed.   During this time the oxygen tension,
with exception of the first reading, varied from 10.7 to 9.1 per
cent.   The CO_2 content did not rise above 0.15 per cent.   The
removal of the animals from the apparatus was rather difficult
taking one and one-half hours, whereby the oxygen rose to 13.8
per cent.   After the injection the animals were returned to the
apparatus together with two larger rabbits, in order to reduce the
oxygen tension more rapidly.   In two hours, when the oxygen
had dropped to 12.3 per cent the reactions were measured.
On the return to the apparatus the oxygen had risen to 14.4
per cent.   In the next four and one-half hours the oxygen dropped
to 8 per cent.   The animals were taken out, the reactions were
measured and the animals returned to 13.1 per cent oxygen which
fell in the course of the next ten hours to 6.4 per cent, while the
CO_2 at the end of this time was 0.24 per cent.   The weights of
the control animals were (1) 600 grams, (2) 600, (3) 630,
(4) 2280.   That of the others (1) 760 grams, (2) 640, (3) 680,
(4) 1960.   The measurements of the reactions in mm. were as
follows:

		1	2	3	4
		mm.	mm.	mm.	mm.
After two hours.........	{ Controls......	Indefinite	29	27	24
	{ Experiment...	42	27	20	33
After seven and one-half hours................	{ Controls......	32	30	45	36
	{ Experiment...	62	35	29	44

The reaction of control 2 was less intense than that of its mate
although after two hours the size of the two reactions was about
equal.   After about twenty hours the reaction of the controls

1, 2 and 3, had diminished very much in intensity while that of 4 had increased. The reaction of 3 had nearly disappeared in the apparatus, 1 and 4 did not show any marked change and in 2 there was an increase. The conclusion from this series of experiments is that on the whole the reactions of the animals exposed to low oxygen tension were somewhat more intense than those of the control.[3]

Two sets of four animals each were used in the second experiment. Here four animals first received their mustard oil injection and were then put in the apparatus at 9.7 per cent oxygen. This time they were taken out after two hours without any difficulty and after measuring the reactions returned to 11 per cent oxygen. They remained in the apparatus three hours. During this time the oxygen dropped to 8.9 per cent rising again at the end to 9.7 per cent. The CO_2 during the first two hours was 0.3 per cent and rose to 0.67 per cent. The weight of the control animals was (1) 2540, (2) 2000, (3) 1660, (4) 1460, that of the others (1) 2450, (2) 1880, (3) 1640, (4) 1120.

		1	2	3	4	REMARKS
		mm.	mm.	mm.	mm.	
Two hours	Control......	28	34	34	32	Control (1) showed very little swelling
	Experiment...	29	39	40	34	
Five hours	Control......	38	41	41	38	
	Experiment...	33	39	53	44	

All the reactions showed about the same degree of elevation, firmness and reddening, so that there was no noteworthy difference in the intensity of the reactions.

[3] At the end of the experiment the animals were rather quiet in the apparatus and the respiration was rapid and jerky. After the animals had been taken out the oxygen tension was 7.1 per cent the CO_2 0.24 per cent. This atmosphere did not support a flame; a candle, an alcohol flame, a benzene flame and a burning match were extinguished. A rabbit (control) of 600 grams and another one of 2280 grams were put in and left there for ten minutes. The animals were quiet and their respiration was somewhat labored. After removal of the animals the atmosphere of the box did not support combustion of the flame.

Again two sets, each of four animals, served for the next experiment. Here the animals were introduced into an atmosphere containing 9.8 per cent O_2. For the greater part of the time the oxygen varied between 7.2 and 8.1 per cent. The CO_2 did not rise above 0.18 per cent. After a sojourn of twenty hours in that atmosphere the mustard oil was injected and the rabbit returned to 10 per cent O_2, the whole procedure of removing the rabbits and measuring the swelling taking not more than 15 minutes. Three hours later with the oxygen at 9.1 per cent the animals were taken out easily the reactions measured and the animals returned for three and one-half hours. The oxygen dropped from 9.8 to 9 per cent, and the CO_2 rose to 0.37 per cent. The controls weighed (1) 2280, (2) 2360, (3) 2380 (4) 2340, the others (1) 2000, (2) 2000, (3) 1940, (4) 1820.

		1	2	3	4
		mm.	mm.	mm.	mm.
Three hours	Controls......	33	36	28	34
	Experiment...	31	22	20	27
Seven and one-half hours	Controls......	35	50	37	40
	Experiment...	35	31	32	40

The exposure to an atmosphere of low oxygen tension had certainly not resulted in more intense reactions, to the contrary, the reactions of the control animals were somewhat more intense. One of the animals (no. 2) had shown marked symptoms about five hours after being put in the apparatus, it had a marked dyspnoea and was staggering. The oxygen was at about 7.5 per cent, the CO_2 at 0.18 per cent. On admitting oxygen (to 8.1 per cent) the animal improved. The next morning with 7.2 per cent O_2 and 0.1 per cent CO_2 the same animal showed lachrymation and marked dyspnoea, while the other animals moved but little and were perhaps slightly dyspnoeic. At the end of the experiment all the animals were well and lively. The exposure to atmospheres with such low oxygen tension does not seem to be tolerated equally by all animals.

In the last series five animals were kept in the apparatus eighty-four hours before being injected with mustard oil. During this time the highest oxygen content was 12.9 per cent, the lowest 9.8 per cent, most of the time it varied but little from 11 per cent. The CO_2 content rose once as high as 2.04 per cent apparently without causing any discomfort to the animals. After the injection the animals were kept in the apparatus five hours before the reactions were measured, the oxygen dropping gradually from 12.6 to 11.25. The taking out required more time (thirty-eight minutes) and the oxygen rose to 12.7 per cent falling during the next five hours to 11 per cent. After measuring the reactions the animals were kept about thirteen hours longer at about 11 per cent O_2. The CO_2 did not rise above 0.53 per cent. The controls weighed (1) 1480, (2) 820, (3) 1500, (4) 1460, (5) 1780. The other animals were weighed before entering the apparatus and at the end of the experiment (1) 1260, 1200, (2) 1100, 1060, (3) 1540, 1360, (4) 1400, 1250, (5) 1640, 1580.

		1	2	3	4	5
		mm.	mm.	mm.	mm.	mm.
Five hours..................	Controls......	32	34	34	34	37
	Experiment...	39	44	32	38	35
Ten and one-half hours.......	Controls......	39	45	38	49	41
	Experiment...	50	62	39	47	41

At the end of the experiment most of the reactions had diminished very much and similarly in both sets.[4] This experiment does not indicate a definite influence of the prolonged previous exposure to a relatively low oxygen tension of the atmosphere on the mustard oil inflammation, one way or another.

Two other methods were tried that promised some interference with oxidations in the organism. Since these experiments were rather of a tentative nature we will refer to them but

[4] During the experiment the temperature in the apparatus did not vary more than 2.2°C.

briefly.   About a third of the calculated amount of blood was withdrawn from two rabbits before the institution of mustard oil in the eye.   In both cases the edema required one and one-half to two hours to reach a marked degree and it did not develop as fully as in the controls.   There was a considerable inhibition.

Four animals were exposed to illuminating gas.   The effects of intoxication with American illuminating gas are according to Yandell Henderson, (14), practically those of CO.   These experiments were done very roughly.   In three animals with more or less severe symptoms of intoxication there was a corresponding inhibition.   One animal that received the mustard oil showed only a little unsteadiness when taken out of the box.   This animal quickly developed a very pronounced reaction but since undiluted mustard oil was instilled in the eye it is very difficult to say whether there was a definite increase, even though the edema had hardly diminished the next day.

A number of animals received intravenous injections of sodium cyanide preceding the instillation of mustard oil in the conjunctival sac.   Since it is somewhat difficult·to distinguish definitely an increase of the reaction when undiluted mustard oil is used, these experiments were made with a drop of mustard oil diluted with an equal volume of olive oil.   With a dilution 1:1 there occurred always a rather marked edema about an hour after the instillation and this dilution had the further advantage that after a lapse of about twenty hours the lesions are as a rule not pronounced, so that the result of a more intense reaction can still be seen at that time.   The sodium cyanide solution was always freshly prepared from a titrated solution of HCN and contained 0.62 mgm. HCN as sodium salt in a volume of 10 cc. the solution being made with saline.

Rabbit 153.   1590 grams.   Received 5.5 cc. NaCN solution, it showed some embarrassment of respiration.   The edema after one hour was decidedly more marked than that of the control and remained so during the time of observation.   The next day the lesions were much more pronounced than those of the control.

Rabbit 154. 1660 grams. 8 cc. NaCN had but slight symptoms. The reaction was even more intense than that of no. 153.

Rabbit 156. 1320 grams. 6 cc. NaCN, had but slight symptoms. Reaction exceeded that of control. Next day lesion much more pronounced than that of control.

Rabbit 158. 850 grams. 6.5 cc. NaCN, had but slight symptoms. Reaction even more pronounced than of no. 156.

Rabbit 159. 1270 grams. 6 cc. NaCN, had but very slight symptoms. The reaction throughout was about like control.

Rabbit 170. 1930 grams. Received first 10 cc. NaCN without any symptoms, then 4 cc. more were injected resulting in convulsions and marked dyspnoea, rapid recovery. The reaction was certainly not less intense than that of the control and perhaps a little more.

Rabbit 172. 2390 grams. 16 cc. NaCN. Convulsions, marked dyspnoea, rapid recovery. The reaction was throughout less intense than that of the control.

In four of the seven experiments the reaction of the animals injected intravenously with NaCN was decidedly more intense than the reaction of their control animals. These animals showed but slight constitutional symptoms such as some embarrassment of respiration and some excitement. In two animals (nos. 159 and 170) the reaction did not differ markedly from their controls;—no. 159 had but very slight symptoms, no. 170 on the contrary showed very marked symptoms, as marked as no. 172 where an inhibition was noted. Taking in consideration the difficulty of finding the proper dosage of this most potent drug for each individual animal it is evident that the intravenous injection of NaCN is capable of intensifying the conjunctival mustard oil reaction of the rabbit. A few experiments were made to see whether it were possible to overcome the inhibition of the mustard oil reaction due to sodium iodoxybenzoate by means of HCN. The solution used contained 1.7 per cent HCN. It is to be noted that the dosage of sodium iodoxybenzoate employed here never failed to produce a most marked lasting inhibition.

Rabbit 131. 1720 grams. Received 17 cc. $\frac{M}{20}$ sodium iodoxybenzoate intravenously, then 1 drop HCN in one eye and 1 drop of mustard oil in the other eye. After two hours there was a considerable edema.

Rabbit 133. 1400 grams. Received 12 cc. $\frac{M}{20}$ sodium iodoxyben-zoate intravenously then 1 drop HCN in one eye and 1 drop mustard oil in the other eye. Two and one-half hours later there was consid-erable edema progressing to marked edema.

Rabbit 134. 1380 grams. Control. 12 cc. $\frac{M}{20}$ sodium iodoxyben-zoate, then mustard oil in eye. After thirty minutes slight edema, hardly progressing any further.

Rabbit 135. 1380 grams. Received 12 cc. $\frac{M}{20}$ sodium iodoxyben-zoate intravenously then 1 drop HCN in one eye and 1 drop mustard oil in the other eye. One and one-half hours later there was a slight edema, the animal received another drop HCN. Thirty minutes later there was considerable edema. Thirty minutes later received another drop HCN. One-half hour later there was not much increase of the edema. The next morning there was considerable edema and secre-tion, hyperemia and opacity of cornea.

Rabbit 136. Control 1400 grams. Received 12 cc. $\frac{M}{20}$ sodium iodoxy-benzoate, intravenously, and a drop of mustard oil in the eye. Thirty minutes later there was a slight edema which had perhaps increased a little seven hours after the injection. The next morning there was some injection, slight edema and the cornea was clear.

Rabbit 81. 1980 grams. Received 13 cc. sodium iodoxybenzoate solution, followed by HCN and mustard oil, it showed a marked edema after two hours.

Rabbit 68. 1040 grams. 10 cc. sodium iodoxybenzoate. HCN and mustard oil. In this animal the result was less pronounced.

Seven other animals treated in the same manner died.

The results indicate that the inhibition caused by the in-travenous injection of sodium iodoxy-benzoate may be overcome to a certain extent by means of hydrocyanic acid.

GENERAL DISCUSSION

The data presented show that the sodium iodoxy- and iodoso-benzoates exhibit their inhibiting effect on the inflammatory edema produced by mustard oil applied to the conjunctiva of the rabbit. This inhibition is just as pronounced as when the oil was administered intracutaneously, as in previously recorded experiments. The fact that various other substances containing chemically active oxygen also exercised such an inhibiting in-

fluence, even if not to the same degree, lends strong support to the conception that the chemically active oxygen is concerned in some way in the activity.

Some of the results obtained by Amberg and Knox (1) pointed rather strongly to the possibility that the sodium iodoxybenzoate may unfold its activity by virtue of an influence exercised on the tissue where the inflammatory stimulus was applied. In these experiments the inflammation was produced by the injection of horse serum intracutaneously in rabbits sensitized to horse serum. Diluting the serum with a sodium iodoxybenzoate solution offered the great advantage of applying the drug exactly where and when it was wanted. There was an unmistakable inhibiting influence. On the contrary when the serum was diluted with sodium cyanide there was an unmistakable intensification of the reaction. From these results the authors arrived at the hypothesis that measures favoring oxidative processes in the tissues tend to inhibit inflammatory processes, while measures interfering with oxidative processes have the opposite tendency. Speaking in such general terms of oxidative processes in tissues may not be deemed justifiable, taking in consideration the number of oxidations which must take place in the organism, requiring for their accomplishment various conditions. Thus Batelli and Stern (15) showed that the oxidation of alcohol, succinic acid and citric acid by tissues is brought about under very dissimilar conditions. However certain conditions may be assumed to inhibit all oxidations in the body. There are differences in the conduct of iodoso- and iodoxy-benzoic acid, the iodoso-benzoic acid (Grove and Loevenhart (9), Jahn (7)) oxidizing phenolphthalin to phenolphthalein, under proper conditions, while the iodoxy-acid does not. Furthermore the sodium salt of the iodoso-benzoic acid yields oxygen much more readily to the perfused isolated mammalian heart (Loevenhart and Eyster (16)) than does the corresponding salt of iodoxy-benzoic acid. If then a substance with chemically active oxygen like ammonium persulfate does not inhibit the mustard oil inflammation, it does not discredit the view that the inhibitory activity of the other substances is due primarily to their chemi-

cally active oxygen. In the experiments where animals were exposed to an atmosphere with diminished partial pressure of oxygen, the reduction of the oxygen was sufficient to elicit some responses from the organism. Dallwig, Kolls and Loevenhart (17), keeping animals in atmospheres of low partial pressure of oxygen, proved that after a certain length of time there is an increase in the haemoglobin and in the number of red blood corpuscles. David (18) describes a hyperemia of the lungs on shorter exposures to 9 to 10 per cent O_2. Von Terray (19), exposed rabbits and dogs to mixtures of hydrogen and air with varying oxygen tension; the experiments lasted thirty-five to ninety minutes. Coincidently with the first visible changes of respiration at 10.5 per cent O_2 he reports a beginning increase in the excretion of lactic and oxalic acid in the urine. Quite remarkable are his experiments where the animals were kept at about 2.7 per cent O_2 from thirty-four to ninety minutes The signs of oxygen want did not develop immediately but after several minutes. The respiration of Strangonow's (20) animals ceased when the oxygen content of the inspired air fell to 3.5 per cent, a figure agreeing well with those given by previous authors. Loewy (21) in his investigations on man points out that the effect of low oxygen tension depends on the oxygen tension of the alveolar air and that with proper breathing it was possible to sustain an alveolar O_2 tension of 5.5 to 6 per cent when the inspired air contained less than 8 per cent oxygen. When the alveolar air contains less than 5.5 per cent O_2 the oxygen consumption begins to decrease and the respiratory quotient to increase, a sure sign of oxygen want. As a rule the oxygen tension of the alveolar air when the inspired air contained 11.5 to 10 per cent O_2 was 6 to 5 per cent. There was, therefore, in all probability an interference with tissue oxidation in our experiments. In spite of this the mustard oil inflammation was not intensified. These experiments do not support the hypothesis that measures interfering with tissue oxidations tend to favor the inflammatory edema. But at the same time they do not disprove it for various reasons. It must be kept in mind that it may be very difficult to bring about the proper degree or kind of interference with

local oxidations in the tissue here concerned by reducing the oxygen supply to the body as a whole.

The investigations of J. Loeb (22) on the physiological action of oxygen want brought to light some very significant facts. Fructified eggs of ctenolabrus and of the sea urchin were kept in a current of hydrogen washing out the oxygen. When the oxygen had been washed out thoroughly there was no more complete cell division. When the oxygen was not washed out sufficiently cell division took place. We have then to consider the possibility that by the time the oxygen supply to a tissue like the skin is sufficiently reduced to effect the oxidations in question the general effect on the organism may offset any influence the diminished oxidations might have exercised on the inflammation. The experiment with cyanide, by Amberg and Knox, and those given in the preceding pages, show an intensifying effect on the inflammatory edema. Under proper conditions it seems possible to overcome to a certain extent the inhibition produced by sodium iodoxybenzoate with hydrocyanic acid.[5] The great advantage cyanide offers over the methods influencing the oxygen supply to the tissues is that it inhibits oxidations no matter how abundant the oxygen supply (Geppert (23)). This being the case we are at present certainly justified in bringing the intensifying effect of the hydrocyanic acid into correlation with its influence on oxidative processes in the tissues.

The experiments with illuminating gas again illustrate the fact previously noted by Amberg and Knox (1) as well as by Januschke (6) that more or less severely intoxicated animals may not react with a full development of an inflammatory edema. Rarely, quite severely poisoned animals may give surprisingly good reactions.

Both animals which had been bled showed an inhibition. But the rapid withdrawal of about one-third of the total amount of blood must have a rather pronounced and complicated effect on the organism, and the acute reduction of the oxygen carry-

[5] Dr. A. D. Hirschfelder was kind enough to notify us that he also has seen a marked intensifying action of an intravenous injection of NaCN on the conjunctival mustard oil reaction of the rabbit.

ing power of the blood in its effect on the tissues may play a very insignificant rôle. Salant and Wise (24) observed no diminution of the power to oxidize citric acid in animals which had been bled. In the effect of bleeding on inflammation, the tendency of the fluid in the tissues to pass into the vessels may enter in consideration.

We tried to obtain some information with regard to the withdrawal of water from the organism, utilizing the result of Woodyatt (25) with continuous intravenous injections of glucose. A rabbit of 1942 grams received glucose at the rate of 5.1 grams per kilogram an hour with Woodyatt's (8) motor syringe, that is, 57.6 cc. of a 40 per cent solution in water in one hundred and thirty-five minutes. After eighty-two minutes the rabbit voided 49 cc. urine, whereupon mustard oil was instilled in the eye. With the first visible change in respiration the injection was stopped. The rabbit had passed another 54 cc. urine and no more could be expressed from the bladder. The weight had dropped to 1890 grams. The edema became fairly marked during the thirty minutes following the instillation and did not differ very much from that of the control. From this time on it hardly progressed while that of the control proceeded as usual. In a second experiment an animal of 1965 grams having passed in the preceding twenty-four hours about 300 cc. of urine received glucose at the rate of about 6.5 grams per kilogram an hour, the total amount injected being 75.7 cc. of a 40 per cent solution The animal passed urine as follows:

	cc.
After 56 minutes	63
After 71 minutes	45
After 88 minutes	31
After 108 minutes	21
After 112 minutes	20
After 119 minutes	16
After 126 minutes	14
After 136 minutes	14
Total	224
Specific gravity	1.024

The mustard oil was instilled when the injection had been going eighty-one minutes and there was hardly any edema, while the experiments lasted. About the time of the last urination changes in the respiration became apparent and when the injection was stopped four minutes later, the animals was sick, shivering intensely and died fifty-one minutes later. But until nearly the end of the injection this animal did not show any symptoms whatever. In these experiments the dehydration of the organism is probably not the only factor bringing about the inhibition, but that it does play a considerable part may be regarded as fairly certain when we take in consideration that Sansum and Wilder (26) were able to reduce the intraocular pressure in cases of glaucoma very considerably by means of such glucose injections leading to a considerable diuresis.

A number of factors exercise an influence on the production of edemas.[6] The data at our command emphasize the possibility that the condition of the tissues brought about by the injury leading to the inflammation plays a rôle in the production of the inflammatory edema. Furthermore within certain limits an influence on tissue oxidations seems to determine an inhibition or intensification of the response to inflammatory stimuli. Such an effect could be most readily. explained on the basis of the theory of Fischer (27) as an influence on the hydration of the tissue colloids. Some obstacles such as the results of Hirschfelder (28) make it impossible to accept this explanation unconditionally. Hirschfelder dropped mustard oil in the conjunctival sac of rabbits, cut out the lids and placed them in Ringer's solution or rabbit's serum, without noticing any swelling.[7]

Januschke (6) studied the effect of various substances on the mustard oil swelling of the rabbit's conjunctiva and on the basis of his results and those of others he attributes the inhibiting effect noted to an influence on an apparatus of inflammation,

[6] See for instance, Wells Chemical Pathology, 1917, Chapter XII, Edema.

[7] There is another possibility of explaining the inhibiting action of the substances containing chemically active oxygen on the one hand and the intensifying one of cyanide on the other by the assumption of an influence on the permeability of the vessel walls. Hereby again an influence on the hydration of the colloids of the vessel walls has to be taken into account.

or at least of exudation of the conjunctiva. This apparatus consists of sensory nerve endings, nerve fibers and blood vessels. Exclusion of the sensory nerve endings by degeneration or by drugs such as narcotics or analgesics inhibit the mustard oil reaction. Substances like calcium and epinephrin are supposed to act on the vessels, possibly by an influence on their permeability. In this scheme of Januschke the beautiful experiment of Bruce (4) is of great importance. The destruction of the ophthalmic branch of the third nerve, when sufficient time for the degeneration of the peripheral part was allowed, inhibited the mustard oil reaction of the conjunctiva completely. Stevenson and Reid (29) were unable to confirm the results of Bruce, neither did their clinical experiments, applying cantharides plaster to anesthetic areas in which the sensory nerve endings have degenerated, support the view that the sensory nerve endings are necessarily concerned in the inflammatory reaction. It seems that an inflammation can occur when the sensory nerve endings are excluded from the field of action. With the exclusion of the sensory nerve endings there remains of the exudation apparatus of Januschke only the vessels. That is we return to the conceptions of Cohnheim (30) who refers the inflammatory edema to an injury of the vessel walls. It is of particular interest to us here that Cohnheim regards this injury as due primarily to a lack of oxygen. The conception toward which we lean is really still older, going back to Virchow (31). It is, that reactions set in motion in the tissues bearing the first brunt of the injury participate in the production of the inflammatory edema.

Vieles ist, wie wir sehen, nicht ein aus den gefässen durch den Blutdruck hervorgepresstes, also passives Exudat, sondern vielmehr, wenn ich mich so ausdrücken soll, Educt oder Extract aus den Gefässen in Folge der Thätigkeit, der activen Anziehung der Gewebselemente selbst.

The chief objection of Cohnheim was that such attractions were unknown to physiology, an objection having lost its force in the course of time.

CONCLUSIONS

The intravenous injection of the sodium salts of o-iodoso- and o-iodoxy-benzoic acid inhibits the mustard oil reaction of the rabbit conjunctiva as well as that of the skin. No other visible changes in the organism accompany this inhibiting action. Other substances containing chemically active oxygen such as the sodium salts of diphthalic peroxide, iodic and periodic-acids have an inhibiting action on the mustard oil inflammation.

It seems reasonable to ascribe the inhibiting action of these substances to the chemically active oxygen.

Exposure of rabbits to atmospheres with reduced partial pressure of oxygen did not have any marked effect on the intensity of the intracutaneous mustard oil reaction.

Intravenous injections of NaCN seem capable of intensifying the conjunctival mustard oil reaction under proper conditions.

Within certain limits an influence on tissue oxidations seems to determine an inhibition or intensification of the response to inflammatory stimuli.

It seems possible to alter the character of the conjunctival mustard oil reaction by intravenous injections of substances, such as sodium hypochlorite.

Irritation produced elsewhere in the organism, as by intraperitoneal injections of kaolin, animal charcoal, mustard oil, may exercise an inhibiting influence on the conjunctival mustard oil reaction of the rabbit.

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THE ACTION OF CERTAIN EMETINE DERIVATIVES
ON AMOEBAE

F. L. PYMAN AND C. M. WENYON

From the Wellcome Bureau of Scientific Research

Emetine has been largely employed for the treatment of amoebic dysentery and has proved to be of great value. It seemed possible, however, that some derivative of emetine or one of the other alkaloids associated with it in ipecacuanha might prove to have a greater toxicity to amoebae relatively to man than the parent substance. This led us to carry out a preliminary survey of the relative action of the alkaloids of ipecacuanha and some of their derivatives on amoebae, from which we also hoped to find some relation between the chemical constitution and the amoebacidal action of these substances.

The substances tested and their formulae are given below:[1]

No. 1. Emetine hydrochloride .... $C_{29}H_{40}O_4N_2$, 2HCl, 7H$_2$O

No. 2. Cephaeline hydrochloride .... $C_{28}H_{38}O_4N_2$, 2HCl, 7H$_2$O

No. 3. N-Methylemetine hydrobromide .... $C_{30}H_{42}O_4N_2$, 2HBr, 3H$_2$O

No. 4. N-Methylcephaeline hydrobromide.... $C_{29}H_{40}O_4N_2$, 2HBr, 1$\frac{1}{2}$H$_2$O

No. 5. N-Methylemetine methochloride.......... $C_{32}H_{48}O_4N_2Cl_2$, 5H$_2$O

No. 6. Rubremetine hydrochloride............... $C_{29}H_{33}O_4N_2Cl$, 6H$_2$O

No. 7. Psychotrine sulphate,................ $C_{28}H_{36}O_4N_2$, H$_2$SO$_4$, 3H$_2$O

No. 8. Hydrochloride B obtained by the oxidation of cephaeline........................ $C_{20}H_{27}O_3NCl_2$, HCl, 5H$_2$O

No. 9. Noremetine hydrochloride .... $C_{25}H_{32}O_4N_2$, 2HCl

No. 10. N-Methylemetinemethine oxalate... $C_{32}H_{46}O_4N_2$, $C_2H_2O_4$, 7$\frac{1}{2}$H$_2$O

The amoebacidal action of these compounds was tested by incorporating their aqueous solutions in the medium upon which the amoebae were inoculated. The medium was an

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[1] Full particulars of the chemical and physical properties of these compounds are given in the papers by Carr and Pyman, J. Chem. Soc. Trans., 1914, cv, 1591, and Pyman, 1917, cxi, 419.

agar mixture (Walker's medium) upon which the amoebae grew
luxuriantly when the drug was absent, whilst the amoeba was
one which had been recovered from water and was of the *Amoeba
limax* type.   Three strengths of drug were used in the media
1/1000, 1/10,000 and 1/100,000.   It was assumed that the
amoebae growing upon the moist surface of the agar in the
Petri dish would come under the action of the drugs in these
strengths.   This would only be true if the slight film of mois-
ture upon the surface of the agar contained neither more nor
less of the drug than the bulk of the medium below it.   In each
case a note was made of the extent of the bacterial (B) as well
as the amoebic (A) growth by examination of the agar surface
under the lower power ($\frac{2}{3}$ inch) of the microscope.

In the tables the degree of growth when any occurs is repre-
sented by the numbers 1, 2 or 3.

First series

SUBSTANCE	STRENGTH					
	1/1,000		1/10,000		1/100,000	
	B	A	B	A	B	A
No. 1....................................	—	—	3	—	3	3
No. 2....................................	—	—	3	—	3	3
No. 3....................................	1	—	3	—	3	3
No. 4....................................	—	—	3	—	3	3
No. 5....................................	2	1	3	3	3	3
No. 6....................................	2	—	3	1	3	3
Control..................................	3	3	3	3	3	3
Days of growth........................	5		4		6	

In this series there was no inhibition of amoebic growth with
dilution of 1/100,000 but four drugs showed complete inhibi-
tion and one a partial inhibition with dilution of 1/10,000.

In the second series the drugs in the first series were again tested
in addition to others.   As no. 4 gave a result in the 1/10,000 dilu-
tion not in accordance with the result in the first series the three
dilutions were again tested with the result recorded below as
Repeat.

Second series

SUBSTANCE	STRENGTH					
	1/1,000		1/10,000		1/100,000	
	B	A	B	A	B	A
No. 1..	1	—	2	—	3	1
No. 2..	1	—	2	—	3	1
No. 3..	1	—	3	—	3	1
No. 4..	2	—	3	3		
No. 4 (Repeat)............................	2	—	2	—	2	2
No. 5..	2	1	3	3	3	1
No. 6..	2	1	2	1	3	2
No. 7..	3	3	2	3	3	1
No. 8..	2	1	2	3	3	1
No. 9..	1	—	2	1	3	3
No. 10.......................................	2	—	2	—	3	1
Control......................................	3	3	3	3	3	3
Days of growth...:.......................	5		5		5	

The above results divided the substances tested into three groups:

1. The salts of emetine, cephaeline, N-methylemetine, N-methylcephaeline and N-methylemetinemethine were approximately equally amoebacidal.

2. N-Methylemetine methochloride, rubremetine hydrochloride, the hydrochloride B, and noremetine hydrochloride were inferior to those of the first group in amoebacidal action.

3. Psychotrine sulphate was much inferior to the substances of group 2.

It may now be of interest to discuss the relation between these results, and the constitution of the substances. The three alkaloids—emetine, cephaeline and psychotrine—which occur naturally in ipecacuanha are closely related. The exact constitution of the nucleus or skeleton of these alkaloids is at present unknown, but it is certainly present intact and fully reduced in emetine, cephaeline, N-methylemetine, N-methylcephaeline, and noremetine, for these substances are interconvertible in a simple manner, differing only in the number of methyl groups attached to the oxygen and nitrogen atoms of the molecule.

$$C_{25}H_{27} \left\{ \begin{array}{l} (OH)_4 \\ NH \\ N \end{array} \right.$$

Noremetine

$$C_{25}H_{27} \left\{ \begin{array}{l} (OMe)_3 \\ OH \\ NH \\ N \end{array} \right.$$

Cephaeline

$$C_{25}H_{27} \left\{ \begin{array}{l} (OMe)_4 \\ NH \\ N \end{array} \right.$$

Emetine

$$C_{25}H_{27} \left\{ \begin{array}{l} (OMe)_3 \\ OH \\ NMe \\ N \end{array} \right.$$

N-Methylcephaeline

$$C_{25}H_{27} \left\{ \begin{array}{l} (OMe)_4 \\ NMe \\ N \end{array} \right.$$

N-Methylemetine

$$C_{25}H_{25} \left\{ \begin{array}{l} (OMe)_3 \\ OH \\ NH \\ N \end{array} \right.$$
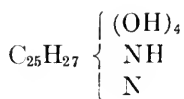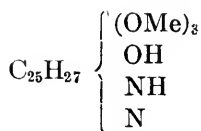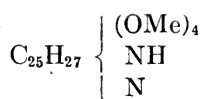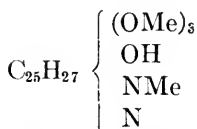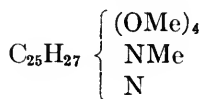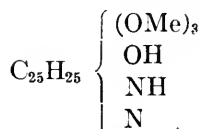
Psychotrine

Noremetine contains four phenolic hydroxyl groups, and is derived from cephaeline or emetine by demethylation. In cephaeline, three of these groups are methylated, being present as methoxy-groups, whilst the fourth remains a phenolic hydroxyl group. Emetine is the O-methyl ether of cephaeline, and contains four methoxyl groups, but no phenolic hydroxyl group. All these compounds contain one tertiary and one secondary nitrogen atom. Methylation of the latter leads in the case of cephaeline and emetine to N-methylcephaeline and N-methylemetine respectively. Of these five compounds, emetine, cephaeline, N-methylemetine and N-methylcephaeline were equal, but noremetine was inferior.

Further methylation of N-methylemetine—a bitertiary base —leads to N-methylemetine methochloride in which both the nitrogen atoms are present in the form of quaternary ammonium salts. This is weaker than N-methylemetine, a result which is not surprising since the quaternary methyl salts of alkaloids frequently differ from the parent tertiary bases and their salts in physiological action. N-Methylemetine methochloride can be converted by chemical treatment into N-methylemetine-methine, a bitertiary base in which two of the nitrogen-rings have been broken. The fact that this substance has apparently the full amoebacidal power of emetine is interesting, but it must be borne in mind that N-methylemetinemethine was tested in the form of its oxalate, and it is possible that the oxalic acid played some part in the action.

Psychotrine contains one phenolic hydroxyl, and three methoxyl groups; it is a secondary-tertiary base, and contains the same nucleus as the other alkaloids, but in an incompletely reduced form. It contains two atoms of hydrogen less than cephaeline, and can be converted into the latter by reduction. The difference in antamoebic properties between the two bases is very striking, cephaeline being one of the most powerful, and psychotrine quite the least of those tested.

Rubremetine similarly differs from emetine in containing less hydrogen, but other constitutional changes may also have taken place in the formation of this compound which is a yellow dye. It proved to be inferior to emetine in antamoebic properties

Finally, the hydrochloride B which was prepared by the oxidation of cephaeline still retains some amoebacidal properties. This is interesting because of the comparatively simple constitution of this substance which is devoid of the guaiacol residue and one of the nitrogen atoms of cephaeline.

ALYPINE, EUCAINE, HOLOCAINE, NOVOCAINE, AND STOVAINE ON THE BLADDER

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I. INTRODUCTORY

While there are numerous references in literature to the action of cocaine on smooth muscle, apparently little attention in this direction has been paid to its synthetic substitutes. Other than in the case of novocaine and holocaine (1), investigation from the standpoint of the smooth musculature seems to have been directed solely to the examination of their effects on the arterioles and on the uveal tract, where in general they are said to produce no reaction or an inhibitory one.

Chemically all of these drugs, except holocaine, consist of a radicle of benzoic acid in combination with a nitrogen containing basic group. Holocaine, on the other hand, is a basic condensation product of paraphenetidin and phenacetin.

The physiology and the pharmacology of the bladder musculature has been investigated by numerous experimentalists; for instance, among others, by Langley and Anderson (2), by Langley (3), and by Jackson (4). In the main, its innervation and reactions correspond to those of the intestinal tract.

Froelich and Loewi (5) and Kuroda (6) have shown that cocaine stimulates the bladder, excised and in situ, producing increase in tone and rhythmicity, an effect just the opposite to that exhibited after epinephrine. From the similarity of chemical structure, it would be expected that the eucaines might act in the same way; but one could hardly reason out the response of the others, particularly that of holocaine.

Further, Froelich and Loewi (5) have shown that cocaine is synergistic with epinephrine on the arterioles and on the bladder, acting apparently as a sensitizer of the receptors through which epinephrine becomes effective. Similar phenomena have been shown in the case of some of the cocaine substitutes on certain other tissues: e. g., on exposed nerves (7) and on blood pressure (8). They have not, however, been definitely shown to potentiate one another (9).

II. METHOD AND SCOPE OF THE INVESTIGATION

It is my purpose in this paper to present the results of a study of the action of several synthetic cocaine substitutes on the excised bladder. Three different species of animals are included: Rats, guinea-pigs, and rabbits. Most of the observations were made, however, on rats; the other species being used chiefly for confirmation of the effects exhibited by the first.

In all cases, the organ was removed under complete chloroform anaesthesia and evacuated of urine. Sections of about 2 mm. in width were then cut from near the greatest circumference and transferred immediately to a bath of oxygenated Tyrode's solution at body temperature.

The suspension method was the one employed in the experiments. The apparatus for the purpose and the details of the procedure have been described in my papers on the internal generative organs of the male (1) and the female (10).

After suspension had been effected, the tissue was weighted just sufficiently to remove confusing tone changes. This was accomplished by placing small aluminum riders on the writing arm of the lever, which had previously been smeared with wax to prevent the possibility of their slipping during the excursions of the lever. The number of these weights necessary to attain the end in view had to be determined by trial for each piece of tissue.

The following are the points to which attention has been given in this investigation: (1) The quantitative and qualitative effects of the several drugs (a) individually and (b) in combination;

(2) the influence of the organ extracts, epinephrine and pituitrin, on their reactions; and (3) their behavior after paralysis of the parasympathetic nerve endings by atropine.

III. DATA FROM SUSPENSION PREPARATIONS OF THE BLADDER

1. Normal. The bladders of all the species examined were rhythmically active when suspended in oxygenated Tyrode's so-lution at body temperature. As a rule, these movements were exhibited from the very beginning of the experiment: occasion-ally, however, from one to four minutes elapsed before they were in evidence.

The rhythm was regular but quicker in some preparations than in others. The amplitude of the contractions was small. Tone changes were not conspicuous and were easily suppressed by appropriate weighting.

2. The cocaine substitutes used individually. The effects of these synthetics were tested on the bladders of each of the species mentioned above. As the reactions of the several drugs[1] were qualitatively almost identical, the gross changes will be dealt with collectively (A) and the fine ones individually (B) as follows:

(A) The gross effects consisted in an increase in the rate, am-plitude, and tone. The weaker concentrations affected chiefly the rate and the amplitude, while the higher also increased the tone. The latent period was relatively short and was approxi-mately the same for all. Continuance of contact with the drug was accompanied by persistence of its effect and withdrawal by prompt return to the original condition. Even the highest con-centrations on prolonged contact failed to produce rigor or ap-parent injury to the tissue.

(B) The individual differences were chiefly quantitative. They will be considered under the caption of the drug in question:

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[1] Orthoform new, anaesthesin, and propaesin were examined in suspensions of from 1:30000 to 1:5000. Anaesthesin and propaesin in the highest concen-trations mentioned produced inhibition and relaxation. Lower concentrations were ineffective. With orthoform new no effect could be obtained. probably on account of its extreme insolubility.
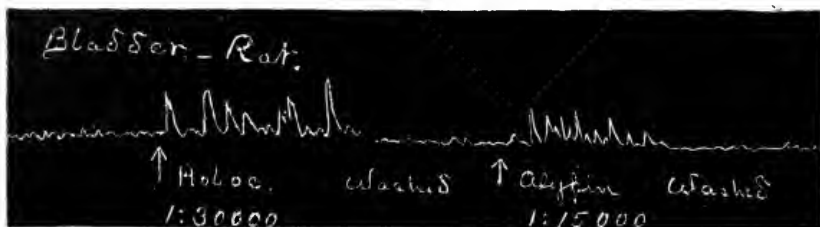
FIG. 1. BLADDER RAT. EFFECTS OF HOLOCAINE AND ALYPINE

(a) Alypine was used in concentrations of from 1: 30000 to 1: 10000. The former dilutions were very effective in the case of the rat, while ones of about 1: 20000 seemed to be the threshold for rabbits and guinea pigs. Compared with cocaine, alypine proved to be approximately one-third as active.
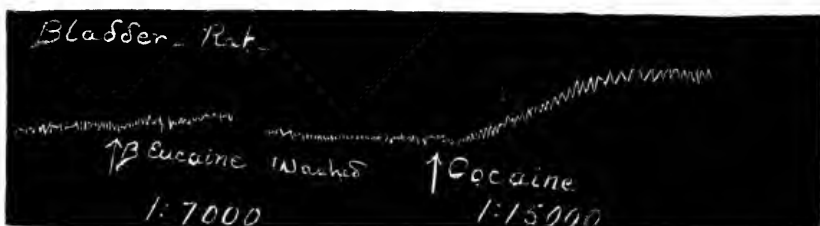
FIG. 2. BLADDER RAT. EFFECTS OF BETA EUCAINE AND COCAINE

(b) Beta eucaine was found to be the weakest of the group in its action on the bladder musculature, being effective only in concentrations of about 1: 5000. Approximately it was one-seventh as active as cocaine. Alpha eucaine, on the other hand,

FIG. 3. BLADDER RAT. EFFECTS OF ALPHA EUCAINE ALONE AND MIXED WITH HOLOCAINE (2: 1)

was a very active stimulant in concentrations even as low as 1:30000. Quantitatively, its effect was somewhat greater than that of alypine. It was approximately one-third to one-half as effective as cocaine.

(c) Holocaine was used in the same concentrations as alypine. It proved to be more active than alpha eucaine, even approaching cocaine itself. Compared with the latter, it was about two-thirds as effective on the bladder musculature.

FIG. 4. BLADDER RAT. EFFECT OF NOVOCAINE AFTER ATROPINE PARALYSIS

(d) Novocaine, next to beta eucaine, was the least active of these synthetics. It was seldom effective in concentrations of less than 1:10000. Quantitatively it was approximately one-sixth of cocaine.

(e) Stovaine was of about the same activity as alypine, being effective in concentrations as low as 1:30000. It would accordingly be about one-third as powerful as cocaine.
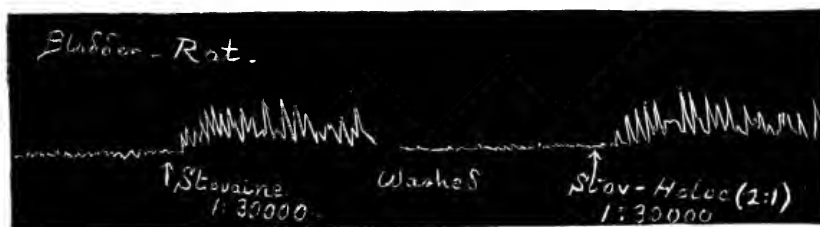
FIG. 5. BLADDER RAT. EFFECTS OF STOVAINE ALONE AND MIXED WITH HOLOCAINE (2:1)

3. *The cocaine substitutes mixed with one another.* Possible potentiation of one of these drugs by another was sought in various ways—prior, subsequent, and simultaneous adminis-

tration in different proportions: for instance, alypine with sto-
vaine (1:1), with alpha eucaine (1:1), and with holocaine (1:1)
and (2:1).   In all cases the result was simple summation.

   4. The cocaine substitutes in relation to epinephrine. Epi-
nephrine produces relaxation and inhibition of the bladder (4,
6, 11).   Cocaine, which on the other hand increases the activity
of the organ, in conjunction with epinephrine enhances its inhibi-
tory action on the intact bladder (5).   Various concentrations of
the synthetic substitutes were tried on my preparations of the
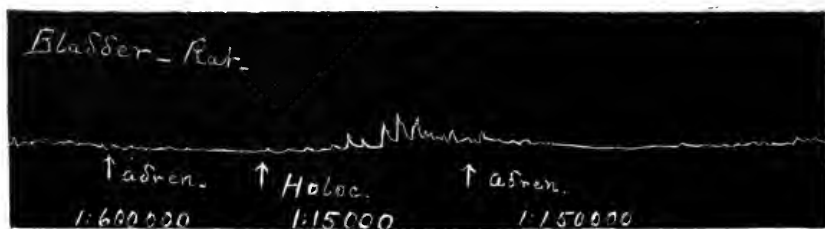
FIG. 6. BLADDER RAT. EFFECT OF EPINEPHRINE AND ANTAGONISM OF
HOLOCAINE
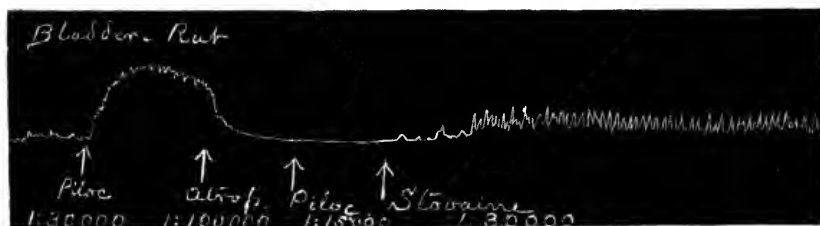
FIG. 7. BLADDER RAT. EFFECT OF STOVAINE AFTER ATROPINE PARALYSIS

excised bladder, at different time intervals in reference to the
application of the epinephrine.   Simple antagonism (algebraic
sum) was the result in every instance.[2]

   5. The cocaine substitutes in relation to pituitrin. Pituitary
extract stimulates the bladder (12).   In a way similar to that
used with epinephrine, possible potentiation for this drug by the
synthetic ones was sought.   The combinations gave simple
summation.

   [2] In my experiments, cocaine itself failed to exhibit any phenomena of syner-
gism with epinephrine on the excised bladder.

6. The cocaine substitutes after atropine paralysis. As in my experiments on the vas deferens (13), examination was made of the effect on the response to these synthetics of paralysis of the parasympathetic apparatus. The procedure in brief was as follows:

(1) Pilocarpine stimulation; (2) atropine antagonism, until several times the originally effective dose of pilocarpine was ineffective; (3) the drug in question; and (4) finally pilocarpine again to determine that the paralysis persisted. In no case was the quantitative or qualitative result altered.

IV. SUMMARY

1. Alypine, alpha and beta eucaine, holocaine, novocaine, and stovaine stimulate the excised bladder when suspended in oxygenated Tyrode's solution at body temperature.

2. Compared with cocaine, the stimulating action of beta eucaine is approximately one-seventh; of novocaine, one-sixth; of alypine, alpha eucaine, and stovaine, one-third; and of holocaine, two-thirds.

3. Mixtures of the synthetic cocaine substitutes with each other give simple summation on the excised bladder.

4. Combinations with epinephrine exhibit simple antagonism and with pituitrin simple summation, on the excised bladder.

5. Paralysis of the parasympathetic myoneural junctions by atropine does not alter the response of the excised bladder to any of the drugs of this series.

6. The conclusion drawn is that these drugs act on the muscle directly, since their effects are the opposite to those of sympathetic stimulation and are unchanged by paralysis of the parasympathetic myoneural junctions.[3]

[3] I have in the course of completion further studies relative to the cocaine substitutes, investigations both of their effects on other tissues and of differences in reaction attributable to differences in chemical structure.

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THE ACTION OF ADRENALIN IN INHIBITING THE FLOW OF PANCREATIC SECRETION

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Benedicenti was the first to observe that adrenalin inhibited the flow of pancreatic juice. He noted that the subcutaneous injection of adrenalin decreased the flow of pancreatic secretion from pancreatic fistulas in dogs for a considerable length of time. Independently, Pemberton and Sweet observed the same action of adrenalin. They studied the effect of injection of adrenalin and extracts of various organs on the flow of secretion from a pancreas stimulated by intermittent injections of secretin. From the results of their experiments they conclude that the inhibition of pancreatic secretion by adrenalin is independent of the rise in general blood pressure, that it is probably specific, and that one of the functions of the adrenals may be an antagonistic action toward the pancreas.

Edmunds repeated the work of Pemberton and Sweet, and studied the action of many other drugs on the rate of pancreatic secretion from a pancreas stimulated by a continuous injection of secretin. He found that practically all drugs or mechanical procedures which increased general blood pressure, decreased the flow of pancreatic juice. However, some drugs, such as adrenalin, produced a greater and more prolonged inhibition of pancreatic secretion than others. Plethysmographic tracings of the pancreas showed that the drugs having the more effective action produced a greater and more prolonged constriction of the pancreatic vessels. Edmunds concluded that adrenalin inhibited pancreatic function by producing anemia of the organ.

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In a research on the general problem of the possible interrelation of the adrenals to the pancreas one of us (Mann) became interested in the action of adrenalin on pancreatic secretion. The results of several experiments dealing with this problem were practically the same as those obtained by Edmunds. However, the work of Edmunds was done before the depressor action of adrenalin had been demonstrated and, in fact, only the large pressor doses of the drug were used in all previous investigations of this subject. It seemed desirable to study the effect of very
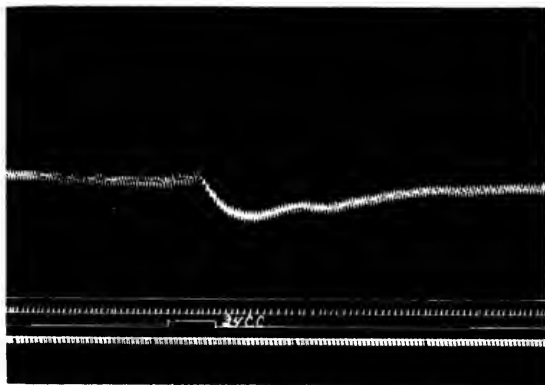
FIG. 1. KYMOGRAPH RECORD OF BLOOD PRESSURE AND RATE OF PANCREATIC FLOW

Time in minutes and seconds. The injection of 1-500,000 adrenalin solution decreased blood pressure from 105 to 75 and decreased pancreatic flow from 0.26 cc. the minute before injection to 0.23 cc. the minute during injection, and 0.20 cc. the minute following injection.

small doses of adrenalin, particularly doses which would produce a depressor action on the blood pressure and on the flow of pancreatic juice.

The animals used in the research were dogs and cats. They were fasted for from eighteen to twenty-four hours before experiments were begun. All the experiments were performed under urethane or ether anesthesia, the latter being administered by means of the Connell apparatus. The ether tension varied between 36 and 44. Carotid blood pressure was recorded

by a mercury manometer. Both vagi were always sectioned. The pancreas was stimulated by secretin prepared by the original method of Bayliss and Starling. In order to obtain a uniform flow of pancreatic juice it was found best to use the continuous injection of secretin as employed by Edmunds. In practice, however, Woodyatt's continuous injection apparatus was found to be of great value. The secretin was injected quite rapidly until the gland began to secrete; it was then decreased to a rate which just maintained a moderate flow of pancreatic juice. In
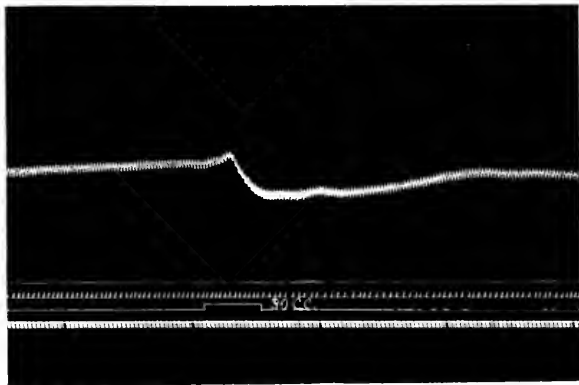
FIG. 2. KYMOGRAPH RECORD OF BLOOD PRESSURE AND RATE OF PANCREATIC FLOW

Time in minutes and seconds. The injection of 30 cc. of 1-500,000 adrenalin solution decreased blood pressure from 105 to 75 and decreased rate of pancreatic flow from 0.27 cc. the minute preceding the injection to 0.27 cc. the minute during the injection, and 0.20 cc. the minute following injection.

this manner it was often possible to have an active gland with a normal general blood pressure. The flow of pancreatic juice was measured by placing in the major pancreatic duct a cannula connected with a glass tube graduated in 0.01 cc. One observer devoted his full time to recording the flow of secretion by means of a signal magnet. When cats were used the adrenalin was administered in exactly the same manner as that employed by Cannon and Lyman in regard to the strength of the solution, the dose and the rate of injection. The technic of injecting with a

buret as Hoskins describes was used in the experiments on dogs. The strength usually employed was 1-500,000 and only fresh solutions were used.

We were not uniformly successful in obtaining the depressor action of adrenalin. This may have been due to several causes, chief of which was the fact that the usual preparation of secretin decreases blood pressure. We were never able to obtain the depressor action of adrenalin when the blood pressure was low, but a pure depressor response was quite frequently obtained with a fairly normal blood pressure. A depressor action preceded by a

Fig. 3. Kymograph Record of Blood Pressure and Rate of Pancreatic Flow

Time in minutes and seconds. The injection of 50 cc. of 1-500,000 adrenalin solution decreased blood pressure from 90 to 70 and decreased rate of pancreatic flow from 0.31 cc. the minute preceding the injection to 0.26 cc. the minute during the injection, and 0.18 cc. the minute following injection. Blood pressure and rate of secretion were both very slow to recover.

slight pressor action was quite commonly secured. The slight initial rise in blood pressure was due at least partially to the quantity of fluid injected.

The action of relatively large doses of adrenalin, injected intravenously at a rapid rate, on the flow of secretion from an active pancreas is very uniform. As the blood pressure suddenly increases, the pancreatic flow is at first slightly increased for a few seconds but soon decreases and it may be completely inhibited. The decrease in pancreatic secretion usually persists a short time after the blood pressure has reached its previous level, and returns to normal under a continuous injection of secretin. Large
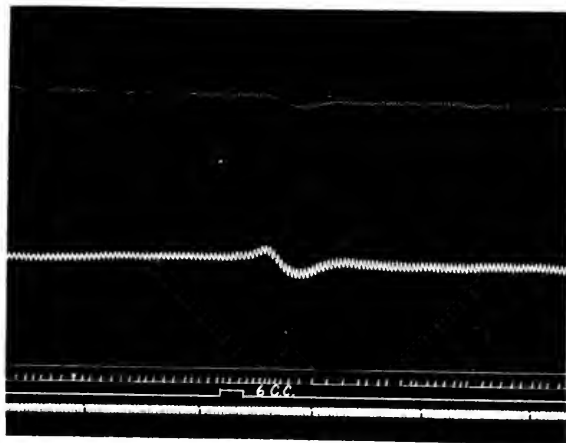
FIG. 4. KYMOGRAPH RECORD OF PANCREATIC VOLUME, BLOOD PRESSURE AND
RATE OF PANCREATIC FLOW

Each was decreased by the injection of 6 cc. of 1-500,000 adrenalin solution.
Time in minutes and seconds.
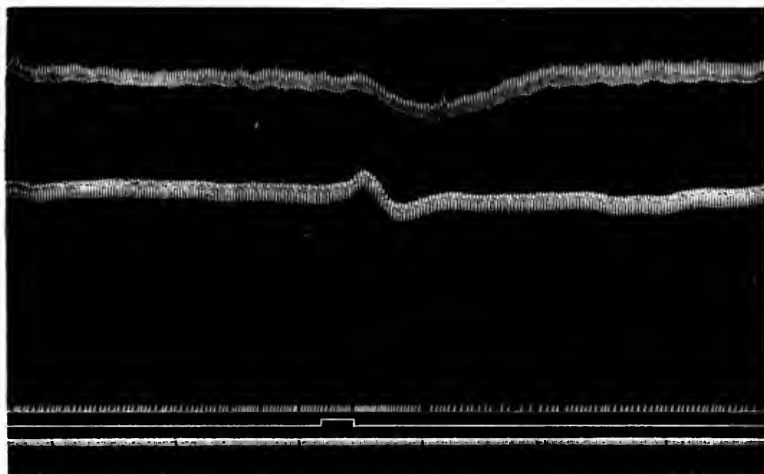
FIG. 5. KYMOGRAPH RECORD OF PANCREATIC VOLUME, BLOOD PRESSURE AND
RATE OF PANCREATIC FLOW

Each was decreased by the injection of 10 cc. of 1-500,000 adrenalin solution.
Time in minutes and seconds.

doses of adrenalin which increase blood pressure 50 mm. or more have never failed, in our experiments, to decrease the flow of pancreatic secretion.

Small does of adrenalin injected slowly, or large amounts of a weak solution of the substance injected rapidly, have a variable action on the flow of pancreatic secretion.   In a very few animals the pancreatic flow was not affected until a dose sufficient to increase blood pressure 40 mm. was injected.   In other animals
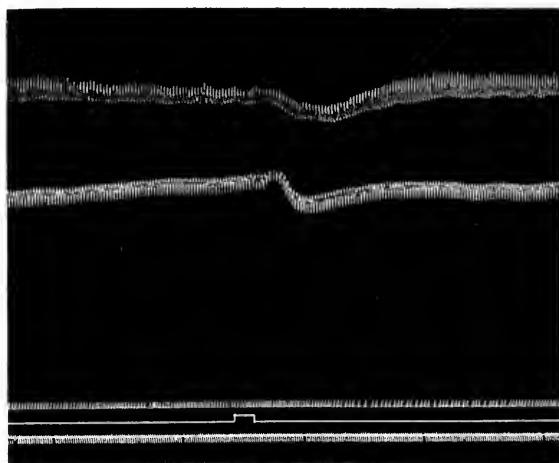
FIG. 6. KYMOGRAPH RECORD OF PANCREATIC VOLUME, BLOOD PRESSURE AND RATE OF PANCREATIC FLOW

Time in minutes and seconds.   Record shows the effect of injection of 10 cc. of 1-500,000 adrenalin solution.   Pancreatic volume blood pressure and rate of pancreatic flow are all decreased.

the pancreas was so sensitive to the action of adrenalin that it was possible, by injecting a very weak solution quite slowly, to decrease the flow of pancreatic secretion without affecting the general blood pressure.

When adrenalin produced a depressor action on blood pressure it usually also decreased pancreatic flow.   In many instances the decrease was quite marked, while in other cases it was slight. . In none of our experiments did adrenalin increase the rate of secretion.

The decrease in the flow of pancreatic secretion secured by adrenalin is not directly dependent on general blood pressure. The fact that by varying the doses and rate of injection of adrenalin it is possible to produce both pressor and depressor response, but only a decrease in pancreatic flow, in each instance demonstrated this fact.   It is also proved by the results of the injection of small doses of adrenalin with a varying general blood
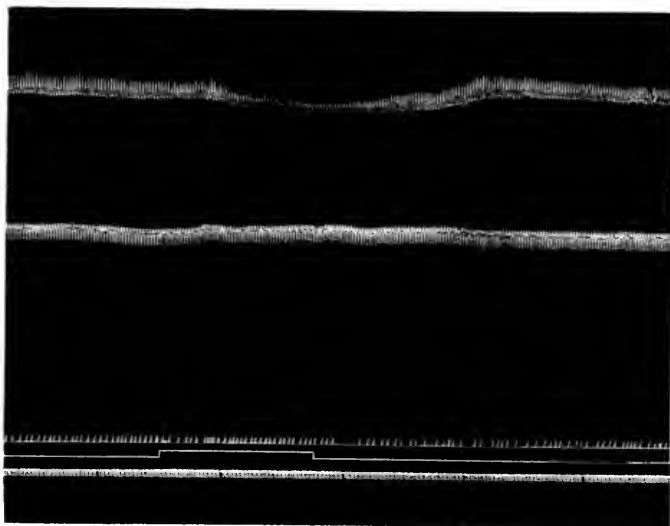
Fig. 7. Kymograph Record of Pancreatic Volume, Blood Pressure and Rate of Pancreatic Flow

Time in minutes and seconds. Record shows the effect of slowly injecting 10 cc. of 1-500,000 adrenalin solution. Pancreatic volume decreased; blood pressure was only very slightly affected; rate of pancreatic flow was noticeably decreased.

pressure. When the injection of a small dose of adrenalin decreases the flow of pancreatic juice at the beginning of an experiment when the blood pressure is relatively high, an equal dose will produce a comparable decrease in flow at the end of the experiment when the blood pressure is low, and the maximum pressure produced may be only half of that produced at the beginning of the experiment.

As Edmunds showed in his work, the probable solution of the problem is secured by a study of pancreatic volume following the injection of adrenalin. By applying a suitable plethysmograph to the pancreas we were able to obtain several records showing the changes of pancreatic volume due to depressor doses of adrenalin. We were not always able to obtain a record of volume changes, owing to technical difficulties, but when we did they always denoted a decrease, viz., a vasoconstriction. Even when adrenalin produced a marked depressor action on general blood pressure, the pancreatic volume and the rate of pancreatic flow also decreased.

SUMMARY

Large doses of adrenalin which produce a marked rise of blood pressure always decrease the flow of pancreatic secretion. Very small doses usually also decrease the activity of the pancreas regardless of whether a pressor or depressor action of blood pressure is produced. Adrenalin also decreases pancreatic volume at the same time it decreases pancreatic flow, regardless of its effect on the general blood pressure. The results of these experiments do not allow us to state definitely that adrenalin does not specifically inhibit the pancreatic cells, but it would seem that the action of adrenalin in inhibiting the pancreatic secretion depends on the amount of blood passing through the gland. Large doses of adrenalin, even though general blood pressure is greatly increased, decrease the amount of blood to the pancreas by excessive local constriction, and thus decrease the flow of pancreatic juice. Small doses may or may not affect the secretion of the pancreas, depending on whether the relation of the local constriction and the changes in general blood pressure change the amount of blood going to the gland. The pancreatic vessels may constrict with a dose which will produce enough dilatation elsewhere to cause a decrease in general blood pressure or which may not produce enough general action to affect blood pressure at all. In every case the cause is the same, a reduction of the amount of blood passing through the gland per unit of time. However, these results all tend to accentuate the fact that the pancreatic

vessels seem to be more sensitive toward the pressor action of adrenalin than those of any other region concerning which we have data.

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THE NON-DEPENDENCE OF THE PROTEIN QUO-TIENT IN THE BLOOD-SERUM UPON THE RAPID-ITY OF METABOLISM WITH ESPECIAL REFERENCE TO THE NON-EFFECT OF ANTIPYRETICS, SODIUM CACODYLATE AND THYROID EXTRACT

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INTRODUCTION

Within recent years considerable attention has been given to the problem of ascertaining the rôle of the serum proteins under physiological and pathological conditions and some significant facts have been reported by various observers. The present study was undertaken with the view of determining the effect of a disturbance of metabolism on the protein quotient when such disturbance is produced by the action of various drugs known to retard or increase the rate of the metabolic processes.

A limited amount of work has been done in this field by previous investigators. Thus Cervello (1), who was the first to engage in this special phase of the work, studied the effects of antipyrin on the serum proteins because this substance tends to retard nitrogen metabolism.

In his first communication this observer reports an invariable marked rise in the percentage of total proteins. This rise was due solely to an increase in the globulin fraction, the albumins remaining normal. He concluded from these observations that the increase in total proteins was due to a retardation of the assimilatory processes as a result of the action of antipyrin. To explain why the increase is confined to the globulin fraction alone, he further assumed that the globulins represent the last stage of

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amino acid resynthesis, and that antipyrin does not interfere with the rebuilding process, but only checks the assimilation of these globulins by the tissues.

In a second report (2), however, the same worker records cases in which the increase in globulins is practically negligible. These negative results are attributed by him to the small size of the doses administered in the later series of experiments. However, considered from the point of view of body weight, the animals employed in the second series of experiments received doses, which were on the whole but very slightly smaller than those first given. In fact, the minimal doses of his earlier experiments were in some cases equal to, and in one instance smaller than, the maximal doses of the later experiments. The two communications of Cervello thus seem to be at variance with each other.

Breinl (3), a second worker in this particular field, undertook the study of the action of antipyrin from a different view point. The work of Moll (4), led this observer to the a priori conception that the rise in globulins reported in Cervello's first communication could be looked upon as being due to a direct chemical transformation of albumins into globulins independently of the rate of the assimilatory processes. How antipyrin could possibly cause such a conversion he does not of course explain.

This investigator, however, obtained what he considered convincing evidence in favor of his hypothesis. He observed an enormous rise in the globulins and what is of great significance to his contention, an accompanying marked fall in the albumins.

In spite of these observations, however, Breinl's hypothesis seems to us to be quite untenable. In the first place, it fails to explain the incongruences between the two reports of Cervello, on the one hand, and the variance of his own results from these on the other. Whereas Cervello with his "larger doses" obtained no change in the albumins and with his "smaller doses" observed practically no effect even on the globulins, Breinl obtained a very marked rise in the globulins and a considerable fall in the albumins with doses less than half as large as the "smaller doses" of Cervello.

Secondly, Moll's theory, upon which Breinl's assumption so largely depends, has itself fallen into disrepute since Gibson (5), has shown that his "globulin" represents nothing else than an intermediate stage in the formation of the alkali metaprotein, while Hammarsten (6), has pointed out that the transformation of albumin into globulin, which Moll and Breinl assume, is impossible since it would involve a synthesis of glycocoll which is not present in the serum albumin.   A further objection to Breinl's hypothesis is that it does not accord exactly with his own observations in that the fall in albumins is very irregular and does not at all correspond with the rise in the globulin fraction as would be expected were the albumins converted into globulins.   One of his five experimental animals, in fact, showed even a rise in the albumins.

In view of these considerations we are disinclined to accept the conclusions drawn by either Cervello or Breinl.   The two communications of the former are mutually contradictory and the views of Breinl face the objections just raised.   Consequently the following questions arise:

1. Does antipyrin actually cause an increase in the percentage of serum globulin?   If so, is this rise accompanied by an equivalent fall in the albumin fraction?

2. Do substances most intimately related to antipyrin in their pharmacological action display a similar property?

3. Do other substances influencing metabolism, (a) in the direction of retardation or (b) in the direction of acceleration, produce any change in the protein quotient?

The present investigation was undertaken with the object of answering these questions.

METHOD AND MATERIALS

For the estimation of the serum proteins Robertson's (7) micro-refractometric method was employed because it is accurate and is at the same time more convenient than the older methods in that very small quantities of blood are required for each determination.   Its use therefore enabled us to make determinations

whenever necessary without in any way disturbing the physiological condition of the animals.

Rabbits were selected as the experimental animals because they are more convenient for frequent bleeding than other available animals.

Determinations were made regularly in all cases through a foreperiod of several days to find the normal variation of each animal. This step is of great importance, since the normal variation of some animals has been shown to amount to as much as 40 per cent. Many of the erroneous conclusions of the earlier workers can be traced to the circumstance that this factor has been underestimated or overlooked entirely.

Four cubic centimeters of blood were drawn for each determination from the marginal auricular vein of the ear into which no injections were made, and the estimations were made on the same day while the blood was fresh.

Whenever possible, the substances were given by mouth, subcutaneously and intravenously. This was considered necessary since in some cases the mode of action of the drug depends upon its route of entrance to the blood stream.

The substances employed were antipyrin (Merck), paramidophenol (Kahlbaum), acetanilide (Kahlbaum), quinine hydrochloride (Merck), sodium cacodylate (Rogers), and thyroid extract (Armour). They were selected from the standpoint of most satisfactorily answering the three questions raised above.

Thus, the action of antipyrin (question 1) was especially thoroughly investigated in view of the contradictory reports of Cervello and Breinl. Acetanilide and paramidophenol were administered for the reason that they are closely related pharmacologically to antipyrin (question 2). Acetanilide is oxidized in the tissues to paramidophenol and this is the active radical to which all the pharmacological properties of acetanilide are probably due.

Quinine was employed because its administration is known to lead to a marked diminution of nitrogenous metabolism, while sodium cacodylate, although chemically unrelated to any of the foregoing substances and differing from them also very widely

in pharmacological properties, nevertheless, is believed to exert à similar retarding influence upon metabolism. Thyroid extract, on the other hand, is admittedly a stimulator of metabolism and was employed as an example of the opposite type of influence upon metabolism to that exerted by the other substances studied.

The dosage has been worked out empirically in most instances. The quantities used in the case of antipyrin, however, were selected with reference to those employed by Cervello and Breinl. In general, the doses were not so large as to interfere appreciably with the health of the animals.

Unless otherwise specified, the drugs were administered about twenty hours before the samples of blood were drawn. In the cases where the immediate effect of a substance was studied, the length of the interval between the administration of the drug and the time of bleeding, is stated in the tables. The following were the results obtained (see tables).

DISCUSSION

Since all of the data are uniformly negative, a lengthy discussion of the experimental results is quite unnecessary. The only noticeable effect of the injections was that the fluctuations in the globulin content were somewhat greater during the period of injection than during the fore-period. In no instance, however, was there a regular, continuous rise or fall in either globulins or albumins. In other words, none of the substances administered have any effect on the albumin and globulin content of the blood-serum.

The non-effect of thyroid extract is confirmatory of the findings of Rowe (8), who observed that in cases of hyperthyroidism the serum proteins remain unchanged. Investigations (Hanson) which will later be published, have demonstrated that such an effective means of altering the rate of metabolism as starving rabbits for a period of as long as six days and subsequent administration of an excess of food results in no change in the relative proportions of the serum proteins.

In the light of our observations, the work of Cervello and Breinl obviously finds no confirmation.

TABLE 1

Rabbit 1. Weight, 2825 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	
May 7........	Normal...............	1.6	4.4	1.1	5.5	20	0.25
May 8........	Normal...............	1.3	3.8	1.0	4.8	21	0.26
May 10.......	Normal...............	1.6	3.8.	1.1	4.9	22	0.29
May 11.......	Normal...............	1.6	4.3	1.4	4.4	23	0.32
May 12.......	Normal...............	1.5	3.8	1.2	5.0	24	0.32
May 13.......	Normal...............	1.5	4.3	1.1	5.4	20	0.26
May 14.......	Normal...............	1.5	4.0	1.1	5.1	21	0.27
May 16.......	Normal...............	1.5	4.2	1.0	5.2	19	0.24
May 17.......	0.2 gram antipyrin in-	1.6	4.2	0.8	5.0	16	0.19
May 18.......	jected intravenous-	1.5	4.5	1.1	5.6	20	0.24
May 19.......	ly daily from May 17	1.5	4.7	1.0	5.7	18	0.21
May 20.......	to May 29 inclusive...	1.5	4.7	1.0	5.7	18	0.21
May 24.......		1.5	5.1	0.5	5.6	9	0.10
May 29.......		1.5	4.35	1.3	5.65	23	0.30
May 29.......	Three hours after ad-						
	ministering subcu-						
	taneously, 1.0 gram						
	antipyrin...........	1.3	4.7	1.3	6.0	22	0.28
June 1........	Normal...............	1.3	5.6 .	0.6	6.2	10	0.17
June 8........	Normal...............	1.5	5.2	0.8	6.0	13	0.15

TABLE 2

Rabbit 2. Weight, 3051 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	
May 7........	Normal...............	1.3	4.5	1.1	5.6	20	0.24
May 8........	Normal...............	1.2	4.0	1.0	5.0	20	0.25
May 10.......	Normal...............	1.3	4.4	1.2	5.6	21	0.27
May 11.......	Normal...............	1.3	4.3	1.4	5.7	23	0.33
May 12.......	Normal...............	1.3	4.3	1.4	5.7	23	0.33
May 13.......	Normal...............	1.3	4.2	1.2	5.4	22	0.28
May 14.......	Normal...............	1.3	4.4	1.4	5.8	24	0.32
May 16.......	Normal...............	1.1	4.4	0.9	5.3	17	0.20
May 20.......	Antipyrin 0.1 gram per						
	os...................	1.3	4.7	1.0	5.7	18	0.21
May 24.......	Thereafter 0.2 gram						
	daily................	1.2	5.2	0.8	6.0	13	0.15
May 29.......	till May 29 inclusive..	1.2	4.2	1.0	5.2	20	0.23
June 1........	Normal...............	1.1	4.7	0.8	5.5	15	0.17

TABLE 3
Rabbit 3.   Weight, 3051 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	
April 11.......	Normal...............	1.2	5.5	0.8	6.3	13.0	0.15
April 13.......	Normal...............	1.5	5.2	1.1	6.3	17.5	0.21
April 16.......	Normal...............	1.5	5.5	1.1	6.6	17.0	0.20
April 18.......	Normal...............	1.4	5.3	1.2	6.5	18.5	0.22
April 20.......	Normal...............	1.5	5.4	1.0	6.4	16.0	0.19
April 21.......	Antipyrin intrave-nously, 0.15 gram.....	1.4	5.3	1.1	6.4	17.0	0.21
April 22.......	Antipyrin intrave-nously, 0.25 gram....	1.4	4.7	1.5	6.2	24.0	0.32
April 23.......	Antipyrin intrave-nously, 0.3 gram.....	1.3	5.0	1.5	6.5	23.0	0.30
April 24.......	Antipyrin intrave-nously, 0.3 gram.....	1.3	5.0	1.5	6.5	23.0	0.30
April 25.......	Antipyrin intrave-nously, 0.35 gram....	1.5	5.6	1.3	6.9	19.0	0.23
April 26.......	Antipyrin intrave-nously, 0.35 gram.....	1.5	5.4	0.8	6.2	13.0	0.15
April 27.......	Antipyrin intrave-nously, 0.35 gram....	1.4	5.1	1.3	6.4	20.0	0.25
April 28.......	Antipyrin intrave-nously, 0.4 gram.....	1.4	4.9	1.9	6.8	22.0	0.38
April 29.......	Antipyrin intrave-nously, 0.4 gram.....	1.5	6.2	1.5	7.7	20.0	0.24
May 24.......	Normal...............	1.2	5.4	1.3	6.7	20.0	0.24
May 24.......	3 hours after injection of 0.5 gram antipy-rin intravenously.....	1.2	5.5	1.4	6.9	25.0	0.25

TABLE 4

Rabbit 4.   Weight, 3000 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	
April 12.......	Normal....	1.5	4.7	1.6	6.3	26	0.34
April 14.......	Normal...............	1.3	4.7	2.0	6.7	30	0.42
April 17.......	Normal...............	1.3	4.2	1.6	5.8	27	0.38
April 19.......	Normal...............	1.3	5.0	1.8	6.8	27	0.36
April 21.......	Antipyrin 0.15 gram intravenously........	1.2	4.6	2.0	6.6	31	0.43
April 22.......	Antipyrin 0.20 gram intravenously........	1.6	4.1	0.8	4.9	17	0.20

TABLE 5

Rabbit 5.   Weight, 1921 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	
May 24......	Normal..............	1.1	4.5	2.1	6.6	32.0	0.46
May 24......	Three hours after 0.2 gram paramido-phenol were injected subcutaneously......	1.1	4.5	1.9	6.4	28.5	0.42

TABLE 6

Rabbit 6.   Weight, 2951 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	
May 7........	Normal..............	1.2	3.5	1.5	5.0	30.0	0.43
May 8........	Normal..............	1.2	4.2	1.7	5.9	29.0	0.40
May 9........	Normal..............	1.3	4.1	1.5	5.6	27.0	0.37
May 10........	Normal..............	1.1	4.1	1.9	6.0	32.0	0.46
May 11........	Normal..............	1.5	3.8	1.9	5.7	33.0	0.50
May 12........	Normal..............	1.3	3.5	1.5	5.0	30.0	0.43
May 13........	Normal	1.3	3.8	1.3	5.1	25.5	0.34
May 14........	Normal..............	1.3	3.6	1.4	5.0	28.0	0.39
May 16........	Paramidophenol subcutaneously, 0.05 gram...............	1.3	4.2	1.0	5.2	19.0	0.24
May 18........	Paramidophenol subcutaneously, 0.10 gram...............	1.3	4.3	1.0	5.3	20.0	0.24
May 20........	Paramidophenol subcutaneously, 0.10 gram...............	1.3	4.45			24.0	
May 24........	Paramidophenol subcutaneously, 0.2 gram...............	1.1	4.0	1.6	5.6	29.0	0.40
May 29........	Thereafter daily 0.2 gram to the 29th, inclusive...............	1.1	3.85	1.4	5.2	.27.0	0.37
June 1.......	Normal..............	1.1	4.9	1.8	5.7	14.0	0.39

TABLE 7
Rabbit 7. Weight, 2951 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	
May 7........	Normal................	1.3	4.8	1.9	6.7	28.0	0.39
May 8........	Normal................	1.5	4.7	2.0	6.7	28.0	0.42
May 9........	Normal................	1.4	4.8	2.0	6.8	29.0	0.41
May 10........	Normal................	1.5	4.6	1.5	6.1	29.0	0.33
May 11........	Normal................	1.5	4.9	1.7	6.6	30.0	0.34
May 12........	Normal................	1.5.	.4.6	1.5	6.1	29.5	0.33
May 13........	Normal................	1.4	4.0	2.0	6.0	33.0	0.50
May 14........	Normal................	1.4	4.7	1.6	6.3	30.0	0.43
May 16........	Paramidophenol sub-eutaneously, 0.05 gram...............	1.4	4.8	1.7	6.5	27.0	0.35
May 18........	Paramidophenol sub-eutaneously, 0.1 gram...............	1.4	4.8	1.7	6.5	26.0	0.35
May 19........	Paramidophenol sub-eutaneously, 0.2 gram...............	1.2	4.5	1.0	5.5	19.0	0.22
May 24........	Thereafter daily to the 29th, inclusive, 0.2 gram...............	1.1	4.7	1.0	6.7	30.0	0.36
May 29........		1.2	4.0	2.35	6.35	37.0	0.58
June 1........	Normal................	1.1	4.6	1.9	6.5	29.0	0.40
June 8........	Normal................	1.2	5.4	2.1	7.5	28.0	0.39

TABLE 8
Rabbit 8. Weight, 1800 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	per cent
May 7........	Normal................	1.2	4.2	1.2	5.4	22	0.30
May 8........	Normal................	1.4	4.8	1.5	6.3	23	0.31
May 10........	Normal................	1.2	3.8	1.2	5.0	24	0.34
May 11........	Normal................	1.5	4.1	1.0	5.1	20	0.25
May 12........	Normal................	1.2	4.4	1.2	5.6	21	0.28
May 13........	Normal................	1.2	4.0	1.1	5.1	23	0.28
May 14........	Normal................	1.2	4.3	1.3	5.6	23	0.30
May 16........	Acetanilide per os 0.2 gram................	1.3	4.4	0.7	5.2	20	0.16
May 17........	Acetanilide per os 0.2 gram................	1.1	3.8	1.4	5.3	27	0.36
May 18........	Acetanilide per os 0.2 gram................	1.3	4.4	1.3	5.7	23	0.30
May 19........	Acetanilide per os 0.2 gram................	1.3	4.4	1.3	5.7	23	0.30
May 20........	Acetanilide per os 0.2 gram................	1.3	4.8	1.5	6.7	30	0.31
May 21........	Died..................						

TABLE 9
Rabbit 9.   Weight, 2825 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	per cent
May 7........	Normal................	1.3	3.7	1.6	5.3	30.0	0.43
May 8........	Normal................	1.2	4.0	1.1	5.1	23.0	0.28
May 9........	Normal................	1.3	3.9	1.2	5.1	23.5	0.31
May 10........	Normal................	1.0	4.2	1.4	5.6	25.0	0.33
May 11........	Normal................	1.1	4.3	1.5	5.8	26.0	0.35
May 12........	Normal................	1.2	3.8	1.2	5.0	24.0	0.31
May 13........	Normal................	1.3	4.2	1.6	5.8	27.5	0.38
May 14........	Normal................	1.3	3.8	1.3	5.1	25.5	0.34
May 16........	Normal................	1.1	4.1	1.2	5.3	23.0	0.30
May 20........	Acetanilide per os 0.1 gram................	1.2	4.6	1.3	5.9	22.0	0.28
May 24........	Acetanilide per os 0.1 gram................	1.1	4.1	1.7	5.8	12.0	0.41
May 29........	Thereafter per os 0.2 gram daily to the 29th inclusive........	1.2	4.6	1.2	5.8	21.0	0.26
June 1........	Normal................	1.1	4.5	1.7	6.2	11.0	0.38

TABLE 10
Rabbit 10.   Weight, 2500 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	per cent
April 11........	Normal................	1.4	5.3	1.9	7.2	26.0	0.36
April 13........	Normal................	1.5	5 5	1.7	7.2	24.0	0.31
April 16........	Normal................	1.4	5.4	1.5	5.9	22.0	0.28
April 18........	Normal................	1.4	5.1	1.7	6.8	25.0	0.33
April 20........	Normal................	1.6	5.9	1.3	7.2	18.0	0.22
April 21........	Quinine subcutaneously, 0.12 gram........	1.6	4.9	1.2	6.1	19.5	0.24
April 22........	Quinine subcutaneously, 0.17 gram........	1.4	4.7	1.5	6.2	29.0	0.32
April 23........	Quinine subcutaneously, 0.25 gram........	1.2	4.9	1.5	6.4	25.0	0.30
April 24........	Quinine subcutaneously, 0.25 gram........	1.2	5.1	1.3	6.4	23.0	0.25
April 25........	Quinine subcutaneously, 0.30 gram........	1.5	4.4	1.4	5.8	24.0	0.32

TABLE 11

Rabbit 11. Weight, 1469 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	per cent
May 24.........	Normal...............	1.1	4.3	2.1	6.4	33	0.48
May 24.........	Three hours after 0.3 gram quinine were injected subcutaneously...............	1.2	4.6	2.1	6.7	31	0.46

TABLE 12

Rabbit 12. Weight, 2486 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	per cent
April 12.......	Normal...............	1.4	3.6	1.8	5.4	33	0.50
April 14.......	Normal...............	1.7	3.9	2.2	6.1	36	0.56
April 17.......	Normal...............	1.4	4.3	1.9	6.2	31	0.44
April 19.......	Normal...............	1.4	4.2	1.8	6.0	30	0.42
April 21.......	Sodium cacoddylate 13 per cent intravenously, 0.2 cc.......	1.1	3.2	2.0	5.2	39	0.62
April 22.......	Sodium cacoddylate 13 per cent intravenously, 0.2 cc.......	1.4	4.7	2.1	6.8	31	0.45
April 23.......	Sodium cacoddylate 13 per cent intravenously, 0.3 cc.......	1.4	5.0	2.2	7.2	32	0.44
April 24.......	Sodium cacoddylate 13 per cent intravenously, 0.3 cc.......	1.1	4.7	2.0	6.7	30	0.42
April 25.......	Sodium cacoddylate 13 per cent intravenously, 0.3 cc.......	1.3	4.8	2.0	6.8	29	0.42
April 26.......	Sodium cacoddylate 13 per cent intravenously, 0.3 cc.......	1.3	4.8	2.3	7.1	32	0.48
April 27.......	Sodium cacoddylate 13 per cent intravenously, 0.3 cc.......	1.3	4.6	2.3	6.9	33	0.50
April 28.......	Sodium cacoddylate 13 per cent intravenously, 0.3 cc.......	1.3	4.0	2.7	6.7	40	0.67
April 29.......	Sodium cacoddylate 13 per cent intravenously, 0.3 cc.......	1.5	4.4	2.3	6.7	35	0.45
April 30.......	Sodium cacoddylate 13 per cent intravenously, 0.3 cc.......	1.4	4.5	2.2	7.7	28	0.46

TABLE 13

Rabbit 13. Weight, 3842 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	per cent
April 12.......	Normal................	1.4	4.8	1.2	6.0	20	0.25
April 14.......	Normal................	1.4	5.4	1.2	6.6	18	0.22
April 17.......	Normal................	1.4	5.2	1.5	6.7	22	0.29
April 19.......	Normal................	1.1	5.4	1.2	6.6	18	0.22
April 21.......	Normal................	1.4	5.1	1.4	6.5	22	0.27
April 22.......	Sodium cacoddylate 13 per cent 0.2 cc intravenously.........	1.3	5.4	1.1	6.5	17	0.20
April 23.......	Sodium cacoddylate 13 per cent 0.3 cc intravenously.........	1.3	5.2	1.3	6.5	20	0.25
April 24.......	Sodium cacoddylate 13 per cent 0.4 cc intravenously.........	1.3	5.6	1.2	6.8	18	0.21
April 25.......	Sodium cacoddylate 13 per cent 0.4 cc intravenously.........	1.3	5.0	1.7	6.7	25	0.34
April 26.......	Sodium cacoddylate 13 per cent 0.4 cc intravenously.........	1.4	5.5	0.9	6.4	14	0.17
April 27.......	Sodium cacoddylate 13 per cent 0.4 cc intravenously.........	1.4	5.7	1.3	7.0	19	0.23
April 28.......	Sodium cacoddylate 13 per cent 0.4 cc intravenously.........	1.4	4.9	1.3	6.2	22	0.27
April 29.......	Sodium cacoddylate 13 per cent 0.4 cc intravenously.........	1.5	5.4	1.4	6.8	20	0.26
April 30.......	Sodium cacoddylate 13 per cent 0.4 cc intravenously.........	1.1	4.6	1.7	6.3	27	0.36

TABLE 14

Rabbit 14. Weight, 3277 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	per cent
May 8.........	Normal................	1.3	4.7	2.1	6.8	31	0.44
May 9........	Normal................	1.2	4.8	2.0	6.1	33	0.39
May 10........	Normal................	1.2	5.7	1.7	7.4	23	0.30
May 11........	Normal................	1.2	5.0	2.1	7.1	30	0.42
May 12........	Normal................	1.3	4.1	2.0	6.1	33	0.48
May 13........	Normal................	1.3	4.9	2.0	6.9	29	0.39
May 14........	Normal................	1.3	4.1	2.3	6.4	36	0.55
May 16........	Normal................	1.4	5.0	2.0	7.0	29	0.40
May 20........	Thyroid extract per os 1 grain. Thereafter 1.5 grains daily to 29th inclusive........	1.4	5.0	1.6	6.6	24	0.32
May 29........		1.1	4.5	1.9	6.4	30	0.42
May 29........	Four hours after 2 grains were administered................	1.3	4.7	1.7	6.4	27	0.36
June 1........	Normal................	1.1	5.2	1.8	7.0	26	0.35

TABLE 15

Rabbit 15. Weight, 2263 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	per cent
May 24........	Normal................	1.3	5.1	1.8	6.9	26	0.35
	Thyroid extract per os 1 grain on the 25th, and 1.5 grains daily thereafter to the 29th inclusive............						
May 29........		1.3	4.5	1.9	6.4	30	0.42
May 29........	Four hours after the administration of 2 grains per os........	1.1	5.1	1.9	7.0	27	0.37
June 1........	Normal................	1.3	5.0	1.8	6.8	27	0.36
June 8........	Normal................	1.3	4.6	1.6	6.2	26	0.34

TABLE 16

Rabbit 16 (control). Weight, 2825 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	per cent
May 7........	Normal................	1.4	4.2	1.0	5.2	19.0	0.24
May 8........	Normal................	1.2	4.2	1.0	5.2	19.0	0.24
May 10.......	Normal................	1.2	4.4	1.0	5.4	18.5	0.23
May 11.......	Normal................	1.2	4.4	1.1	5.5	19.5	0.24
May 12.......	Normal................	1.5	4.1	1.0	5.1	19.5	0.24
May 13.......	Normal................	1.3	4.5	1.1	5.6	20.0	0.24
May 14.......	Normal................	1.2	4.2	1.0	5.2	19.0	0.24
May 16.......	Normal................	1.3	4.6	0.9	5.5	16.0	0.20
May 18.......	Normal................	1.4	3.9	1.4	5.3	26.0	0.35
May 19.......	Normal................	1.3	4.7	1.0	5.7	18.0	0.21
May 20.......	Normal................	1.3	4.4	1.5	5.9	25.0	0.34
May 24.......	Normal................	1.1	5.5	1.2	5.7	17.0	0.22
May 29.......	Normal................	1.3	4.5	1.2	5.7	21.0	0.26
June 8........	Normal................	1.3	4.6	1.1	5.7	20.0	0.24

TABLE 17

Rabbit 17 (control). Weight, 3955 grams

DATE	TREATMENT	NON PROTEIN	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN OF TOTAL PROTEIN	PROTEIN QUOTIENT
		per cent	per cent	per cent	per cent	per cent	per cent
April 11.......	Normal................	1.8	5.6	1.55	7.5	22.0	0.29
April 13.......	Normal................	1.4	5.3	2.1	7.4	28.0	0.40
April 16.......	.Normal................	0.9	6.2	2.2	8.4	26.0	0.35
April 18.......	Normal................	1.4	5.5	1.8	7.3	25.0	0.34
April 20.......	$\frac{M}{6}$ NaCl intravenous-ly, 10 cc............	1.7	5.3	1.9	7.2	26.0	0.36
April 22.......	$\frac{M}{6}$ NaCl intravenous-ly, 10 cc............	1.6	5.8	1.1	6.9	23.0	0.20
April 23.......	$\frac{M}{6}$ NaCl intravenous-ly, 10 cc............	1.4	5.3	1.5	6.8	22.0	0.29
April 25.......	$\frac{M}{6}$ NaCl intravenous-ly, 10 cc............	1.7	5.0	1.8	6.8	26.5	0.36
April 26.......	$\frac{M}{6}$ NaCl intravenous-lý, 10 cc............	1.4	5.7	2.5	8.2	32.0	0.43
April 27.......	$\frac{M}{6}$ NaCl intravenous-ly, 10 cc............	1.3	4.8	2.2	7.0	31.0	0.45
April 28.......	$\frac{M}{6}$ NaCl intravenous-ly, 10 cc............	1.5	5.7	2.5	8.2	30.0	0.43

CONCLUSIONS

1. Antipyrin, even in large doses, causes no change in the serum proteins.

2. Substances most intimately related to antipyrin in their pharmacological action (acetanilide and paramidophenol) have no effect on the protein quotient.

3. Other substances influencing metabolism: (a) In the direction of retardation (quinine and sodium cacodylate), or (b): In the direction of acceleration (thyroid extract) produce no change in the relative proportions of the serum proteins.

These experimental results consistently indicate that the quantity of albumins and globulins in the blood-serum is quite independent of the rapidity of the nitrogenous metabolism.

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NOTE ON THE PHYSIOLOGICAL ACTION OF CORDYCEPS SINENSIS

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Cordyceps Sinensis is a fungus, parasitic upon a caterpillar. It is called by the Chinese Hia-tsao-tong-tchong or Hia tsaon-taon-chung, meaning "Summer-plant-winter-worm" and is supposed by them to be a very effective drug for general debility and to have properties in general like ginseng. It is spoken of by du Halde (1). Jonathan Pereira (2) describes the fungus and states that the drug grows in Thibet and is scarce, black, old and rotten specimens costing four times their weight in silver. The same author giving the method of employment of the drug says: "The belly of a duck is stuffed with about 5 drachms of the insect fungus and the bird roasted by a slow fire. When done the insect fungus is removed, its virtue being supposed to have passed into the flesh of the duck. The latter is to be eaten twice daily for eight or ten days."

The close relationship of the fungus to ergot, and the belief of the Chinese that it has properties in general like those of ginseng, indicate that a study of its physiological action might disclose facts of value. When, therefore, Mr. F. N. Meyer brought some of the drug back from China advantage was taken of his kind offer to submit the material for toxicological examination. Prof. Roland Thaxter very kindly identified the fungus as an immature state of Cordyceps Sinensis and furnished considerable other information bearing upon its use in China.

The drug submitted by Mr. Meyer consists of dried caterpillars with a fungus or mushroom growing out of each specimen. About twelve of these individuals are tied together in

a bundle—apparently the form in which the drug comes into commerce.   Each individual, consisting of the fungus and the long, yellow cylindrical body of the caterpillar is about 3 inches long.   The head, segments, legs and other external structures of the caterpillar are easily recognizable.   From the back of the neck grows the slender club-shaped fungus about $1\frac{1}{2}$ inches in length and black in color.

The amount of material available was too small to permit of any extended chemical or pharmacological investigation of the drug at this time.   Nevertheless, the results are recorded here as they may prove of interest to those who may have the opportunity of examining larger amounts of the material.

The results obtained may be summarized as follows: No basic substances other than ammonia soluble in alcohol or Prollius[1] solution were detected.   Aqueous extracts showed no hemolytic action on rabbit's blood.   As the amount of material tested was small these findings can not be regarded as final. The aqueous extract of 1 gram of fungus injected intravenously into a rabbit proved fatal in one case in an hour and a quarter, in another in three hours and a quarter.   For lack of material the minimum lethal dose could not be determined.   However, extracts made by heating with water or alcohol apparently had no effect as the animals survived and showed no symptoms. It is evident that heat destroys the toxicity and if the drug's toxicity is responsible for the curative properties ascribed to the drug by the Chinese their method of administering by cooking as described by Pereira would probably nullify its effects. The fructification of the fungus outside the body of the caterpillar is toxic.   Whether any element of the body of the caterpillar containing no fungus is toxic was not determined.

EXPERIMENTAL

The action of unheated aqueous extracts

0.155 gram of the portion of fungus growing outside the caterpillar body was broken up in a mortar and extracted with 3 cc. water,

[1] This solution contains: ether, 88 parts; alcohol saturated with ammonia, 4 parts; absolute alcohol, 8 parts.

filtered, and the slightly acid filtrate, after neutralizing with sodium carbonate, used for injection.

January 8. . 3.35 p.m.   A white mouse received subcutaneously 0.8 cc. of the above extract.   No immediate effects were noticeable except the apparent discomfort from the relatively large volume of fluid injected.

January 9.   3.00 p.m.   Mouse was in a half stupid state.

January 10.   9.00 a.m.   The mouse was found dead.

One gram finely ground fungus without the caterpillar was digested one hour at room temperature with 5 cc. distilled water, then filtered by suction through pulp.   After releasing the suction the residue was moistened with 2 cc. water and again sucked dry.   The clear filtrate, measuring a little more than 4 cc. was used for injection.

July 28, 1917.   Rabbit 2568, weight 1485 grams, received intravenously 3.6 cc. at 12.00 m.   At first there were no marked symptoms. The rabbit on being released hopped about in the usual manner and was put in a cage.   After a half hour the rabbit was huddled in a corner of the cage and on being disturbed would crawl back into the corner and remain quiet.   No weakness was noticeable at this time but the animal appeared to become stupid, the eyes being half closed. Then respiration and the heart beat became more rapid.   Shortly before death tetanic convulsions set in following each other in rapid succession, and soon growing weaker.   Death occurred at 1.15 p.m. with opisthotonus.

One gram finely ground fungus without the caterpillar was treated as above and filtered, the filtrate this time being neutralized with sodium carbonate which produced a slight precipitate.   This was filtered: 4 cc. of the filtrate were available for injection.

July 30.   Rabbit 2577, weight 1530 grams, received intravenously 4 cc. neutralized extract at 12.15 p.m.   The symptoms of this rabbit at the time of death were the same as those of rabbit 2568 excepting that they developed more slowly.   The same convulsions, gasping, etc., occurred, with death at 3.30 p.m.

The action of heated extracts

0.9 gram of the entire growth, caterpillar and fungus, was pulverized, extracted with water on steam bath and filtered.   The clear filtrate, which was acid to litmus, was evaporated to about 3 cc., neutralized with sodium carbonate and 2 cc. used for injection.

January 10. 11.31 a.m. Rabbit (female Belgian weighing 1430 grams received intravenously 2 cc. above extract. No effects.

1.65 grams of the entire growth, caterpillar and fungus, were extracted by boiling with alcohol. The alcoholic extract was filtered and evaporated to small volume. On standing this deposited by spontaneous evaporation a mass of small crystals mixed with fat. The crystals were dissolved in water and the solution shaken with ether to remove fat. The aqueous layer was separated, reduced to convenient volume on the steam bath, neutralized with sodium carbonate and used for injection.

The crystals resembled those of ammonium chloride and evolved ammonia when heated with alkali.

January 27. 2.21 p.m. Rabbit (female Belgian) weighing 1785 grams received intravenously 2 cc., equal to about one-half the total extract from 1.65 grams caterpillar and fungus. No effects.

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THE FATE OF STRYCHNIN IN THE BODY

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The present investigation was undertaken with a view to determining the approximate rate of elimination of strychnin from the body, and the mechanism of the elimination, whether it be by fixation in one or more of the tissues in an innocuous form, by decomposition, or by excretion. The studies were made on cats, dogs, and guinea-pigs.

The advantages of biologic tests in making quantitative determinations of strychnin in impure solutions are recognized by pharmacologists, and owing to the well known difficulties of extracting this alkaloid from animal tissues and crystallizing it without serious loss, we have used this method, employing the cat chiefly as the test animal for estimating the amounts of strychnin recovered from tissues and excreta. In a few cases where negative results were anticipated we injected urine without other purification than that by filtration, or by evaporation with precipitation of the salts by alcohol and filtration. The presence of ammonia, which may induce strychnin-like convulsions, was considered, but in no case did it prove a disturbing factor.

The toxicity of strychnin varies considerably with different animals; with different modes of administration in the same animal; and with different concentrations of solution and different rates of entrance into the blood stream, but when these points are understood the biologic tests on cats, dogs, guinea-pigs, and frogs can be utilized for quantitative determinations with fairly accurate results. Some batches of frogs are almost useless for the purpose, others afford far more accurate results

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than mammals.   Frogs have the disadvantage of requiring standardization at frequent intervals, so that their use becomes time-consuming and unsatisfactory for work that extends over a period of more than a year as ours has done.   Cats, dogs, and guinea-pigs are far more convenient in such cases, but their use demands careful consideration of the factors mentioned.   In a few cases where extremely small amounts of strychnin were sought frog tests were employed, for while these are often unsatisfactory for quantitative determinations they afford extremely delicate qualitative tests.

We do not claim that we are able to determine the exact amounts of strychnin in tissues and excreta in this way, but the method is far more accurate than the chemical in many cases, and the time that would have been consumed in obtaining the strychnin in crystallized form in so many experiments would have been absolutely prohibitive, and in some cases it would have been impossible where only minute amounts were present. The alkaloid is not very insoluble in water, relatively to the small amounts employed, and alkaline solutions must be shaken with many successive portions of chloroform, or other solvent, in order to extract the last fraction of the alkaloid, and even then some loss is unavoidable, and in the repeated purifications necessary before the pure alkaloid is obtained the loss is often very considerable.

There have been many investigations of the subject of the fate of strychnin in the body, but there is still a want of agreement as to whether all of that administered is eliminated unchanged in the urine, whether elimination is slow or rapid, or whether the poison is fixed' in the liver or other organ and decomposed in part, or slowly given up for excretion.

This confusion has arisen partly from too broad generalizations from the results of experiments on a single species of animal, for the rate of elimination varies widely with different species.   We believe that our results throw some light on the fate of strychnin in the human body, for while we have observed such differences in the rates, the mode of elimination is probably the same in the several species, at least the evidence points in that direction.

The literature relating to the subject is extensive and we shall limit our references mainly to those papers that bear immediately on the problem. A comprehensive bibliography down to 1906 is given by Kobert in his Lehrbuch der Intoxikationen, 1906, pp. 1162–1164, and various text-books, especially Sollmann's Manual of Pharmacology, 1917, review the literature.

I. TOLERANCE

The simplest means of determining the approximate rate of elimination of a poison from the animal body, at least insofar as its presence in an active form is concerned, is by determining the maximum frequency with which a given percentage of the fatal dose can be administered for prolonged periods in such a way as to secure prompt absorption into the circulation without causing death. Soluble salts of strychnin are absorbed promptly and completely from the subcutaneous tissues of the cat, dog, and guinea-pig, as well as from the alimentary canal of the cat and dog, and somewhat less promptly from the alimentary canal of the guinea-pig.

We have administered large doses of strychnin to these three species of animals at frequent intervals, and in some cases have repeated the administration on successive days. The size of the dose and the frequency of repetition were often governed by the condition of the animal during the course of the experiment, the effort being in those cases to give as much of the poison as possible without causing death. Death actually resulted in several instances after a few doses had been given, such accidents being indicated in the protocols of experiments or in the tables. We have also used dogs which had been previously poisoned, some with phosphorus, some with chloroform, the reasons for which will be explained later.

The toxicity of strychnin is fairly uniform when it is administered in uniform concentration and in a given way to animals of any one species. The fatal dose for the cat (in terms of the alkaloid) is about 0.25 mgm. per kilogram of animal when a solution of 1 part in 1000 parts of normal salt solution is injected intramuscularly; about 0.3 mgm. per kilogram when a solution of 1 to 10,000 is used in this way, and about 0.35 mgm. per

kilogram when such a solution is administered through a stomach tube; dogs are slightly more tolerant than cats, the corresponding doses being about 20 per cent larger, and the guinea-pig is very much more resistant, requiring doses of about 3.5 mgm. per kilogram by subcutaneous injection and about ten times as much by the stomach.

Curiously, the fatal dose of strychnin varies more with intravenous injection with variations in the conditions of administration than by intramuscular injection, far less being required to cause death when concentrated solutions are injected rapidly than when dilute solutions are injected slowly.

Anesthetics such as chloroform, ether, and chloretone, interfere so greatly with the actions of strychnin that their use is inadmissible when quantitative estimations of the alkaloid are made biologically.

As little as 0.005 mgm. of strychnin suffices to induce hyperexcitability in a grass frog weighing 10 grams as a rule.

The protocols of several experiments will be given in brief, and the results of all experiments of this type will be tabulated, after which the results will be discussed in some detail. All doses are expressed in terms of the alkaloid but 1.28 mgm. of the sulphate were actually used in every case for 1 mgm. of the alkaloid stated.

Experiments on tolerance

Cat 1; female; weight 2.32 kilos; all injections were made intramuscularly; doses are stated in milligrams of alkaloid per kilogram of weight.

8.14 a.m.   0.25 mgm.
10.14 a.m.   0.1 mgm.
12.14 p.m.   0.1 mgm.
2.14 p.m.   0.15 mgm.
4.14 p.m.   0.1 mgm.
  Following day animal about normal.
8.38 a.m.   0.2   mgm.
10.38 a.m.   0.1   mgm.
12.38 p.m.   0.1   mgm.
2.38 p.m.   0.1   mgm.
4.38 p.m.   0.1   mgm.

A total of 1.3 mgm. per kilogram of weight, or about five times the single fatal dose, was administered within a period of thirty-two hours.

The following day the cat appeared to be normal.

Cat 12; male; weight 2.94 kilos. Doses of 0.2 mgm. of the alkaloid per kilogram of weight were administered through a stomach tube at the hours indicated except where shown by an asterisk, in which case doses of 0.15 mgm. were given.

First day	9.30;	11.15;	1.00;	3.30.
Second day	9.00;	11.00;	1.00;	3.00; 5.00.
Third day	9.00;	11.00;	1.00.	
Fourth day	9.00;	1.30;	4.30.	
Fifth day	9.15;	11.00;	1.00;	3.30.
Sixth day	9.00;	11.00;	1.30.*	
Seventh day	Sunday; no strychnin administered.			
Eighth day	9.00;	11.00;	1.00;	3.30.
Ninth day	9.00;	11.00;	1.00;	3.30.
Tenth day	9.00;	11.00;	1.00;*	3.30.*
Eleventh day	9.00;	11.00;	1.30;*	3.30.*
Twelfth day	9.15;	11.00;	1.30;*	3.30.*
Thirteenth day	9.00;	10.30;	1.00;	3.45.

Cat 12 showed marked hyperexcitability during the greater part of the experiment, and tetanus occurred at times, necessitating a reduction in the succeeding dose. The animal received a total of 26.6 mgm. of strychnin, or 9 mgm. per kilogram of weight, the equivalent of about twenty-five times the single fatal oral dose.

Dog D; female puppy; weight 2.34 kilos. All doses were administered subcutaneously; they are expressed in milligrams of the alkaloid per kilogram of weight.

July 6.	3.10 p.m.	0.25 mgm.
	3.45 p.m.	Hyperexcitable; some spasticity.
July 7.	9.06 a.m.	0.25 mgm.
	1.05 p.m.	0.25 mgm.
	1.28 p.m.	Convulsions.
	4.09 p.m.	0.16 mgm.
	5.15 p.m.	Nearly normal.
July 8.	9.07 a.m.	0.23 mgm.
July 9.		Sunday; no alkaloid administered.

July 10.   9.06 a.m.   0.23 mgm.
           10.06 a.m.   0.23 mgm.
           10.15 a.m.   Severe, and repeated, convulsions; artificial res-
                         piration and anesthetics at intervals.
           12.40 p.m.   Reflexes normal.
            1.06 p.m.   0.16 mgm.
            2.06 p.m.   0.08 mgm.
            3.06 p.m.   0.08 mgm.
            4.06 p.m.   0.08 mgm.
            5.10 p.m.   Appears normal; remained so.

Dog D received a total of 0.86 mgm. of the alkaloid per kilogram of weight in a period of seven hours on the last day of the experiment, and a total of 2 mgm. per kilogram, the equivalent of five times the single fatal dose, was given within a period of four days (ninety-seven hours).

A dog that was nearly moribund from phosphorus poisoning and two in advanced chronic chloroform poisoning were tested for their tolerance toward repeated doses of strychnin. One of the animals that had been poisoned with chloroform had fasted for forty hours previous to the test, and it succumbed shortly after the administration of the fifth dose, and a total of only 0.5 mgm. per kilogram of weight, administered within a period of seven hours. A normal animal that had fasted for forty hours had severe tetanus after the subcutaneous injection of a dose of 0.2 mgm. per kilogram. The protocol of the test of the tolerance of the other dog that had been poisoned with chloroform is given in brief.   •

Experiment on tolerance of dog toward strychnin during chloroform poisoning

All doses of strychnin were injected subcutaneously, and are expressed in milligrams of the alkaloid per kilogram of weight.

Male dog; weight 5.48 kilos.

The animal received a total of 10 cc. of chloroform mixed with 190 cc. of water through a stomach tube in a period of ten days. At the end of this period it was lively but weak, and the weight was reduced to 5.18 kilos. It was then used for the test.

July 5.   8.30 a.m.   0.2 mgm.; followed by hyperexcitability.
              9.30 a.m.   0.1 mgm.; followed by tetanus.
           11.30 a.m.   0.1 mgm.
           12.30 p.m.   0.1 mgm.
             2.00 p.m.   0.1 mgm.
             3.15 p.m.   0.1 mgm.
July 6.   9.30 a.m.   0.2 mgm.; followed by tetanus.
           11.30 a.m.   0.1 mgm.
             1.15 p.m.   0.1 mgm.
             2.30 p.m.   0.1 mgm.
             4.30 p.m.   0.1 mgm.; not followed by tetanus.

Tetanus did not occur after the last dose on the second day and a total of 1.2 mgm. per kilogram of weight in thirty-two hours (about three times the single fatal dose) though it did follow the second dose and a total of 0.3 mgm. per kilogram of weight on the first day. It is clear that a dog suffering from severe chloroform poisoning may eliminate strychnin almost, or quite as fast as the normal dog.

A dog that had received a total of 5 mgm. of phosphorus subcutaneously in a period of three days, and which was very feeble withstood the subcutaneous injection of eight doses and a total of about 0.75 mgm. of strychnin in a period of seven hours and showed less hyperexcitability after the last dose than it exhibited after the second, and a total of only 0.4 mgm. per kilogram, after which tetanus actually occurred.

Experiments on tolerance of the guinea-pig toward strychnin

All doses are expressed in milligrams actually administered—not per kilo of weight.

Guinea-pig 1; female; weight 643 grams.

All doses were injected subcutaneously.

August 15.   9.40 a.m. 1.0 mgm.
            10.31 a.m. 1.0 mgm. Moderate increased reflex ex-
            11.31 a.m. 1.0 mgm.   citability after each dose; mark-
            12.28 p.m. 1.0 mgm.   edly increased after the fourth.
             1.44 p.m. 1.0 mgm.
             2.29 p.m. 1.0 mgm.
             3.00 p.m. 1.0 mgm.
             3.40 p.m. 1.0 mgm.

August 16.	9.26 a.m.	1.0	mgm.	
	9.56 a.m.	1.0	mgm.	
	10.51·a.m.	1.0	mgm.	Moderate increased reflex ex-
	11.51 a.m.	0.5	mgm.	citability essentially as on
	1.00 p.m.	1.0	mgm.	previous day.
	1.30 p.m.	0.5	mgm.	
	2.00 p.m.	1.0	mgm.	
	3.00 p.m.	1.0	mgm.	
	3.26 p.m.	1.0	mgm.	
August 17.	9.25 a.m.	1.5	mgm.	
	10.00 a.m.	0.5	mgm.	
	10.30 a.m.	1.0	mgm.	Conditions essentially as on
	11.00 a.m.	0.5	mgm.	previous day.
	11.45 a.m.	1.0	mgm.	
	12.30 p.m.	1.0	mgm.	
	1.00 p.m.	1.0	mgm.	
	1.30 p.m.	0.5	mgm.	
	2.00 p.m.	1.0	mgm.	Muscular twitchings, repeated
	2.40 p.m.	0.5	mgm.	after all doses following this.
	3.15 p.m.	0.5	mgm.	
	3.45 p.m.	1.0	mgm.	
August 18.	9.20 a.m.	2.0	mgm.	
	10.00 a.m.	1.0	mgm.	Followed by hyperexcitability.
	10.40 a.m.	1.0	mgm.	Followed by hyperexcitability.
	11.20 a.m.	1.0	mgm.	Followed by twitching.
	12.00 m.	0.75	mgm.	Followed by twitching.
	1.00 p.m.	0.75	mgm.	Followed by twitching.
	1.50 p.m.	1.0	mgm.	
	2.00 p.m.			General convulsions.
	2.30 p.m.			Death.

Guinea-pig 6; male; weight 317 grams.
All doses administered orally.

September 6.	12.51 p.m.	9.5	mgm.	
	2.51 p.m.	6.35	mgm.	
September 7.	9.15 a.m.	9.5	mgm.	
	11.15 a.m.	3.2	mgm.	
	1.15 p.m.	6.35	mgm.	
	3.45 p.m.	6.35	mgm.	
	4.15 p.m.			Found dead.

The condition of guinea-pig 1, after the sixth dose of the last day indicated that it would have survived had no more been given. In this case an amount equal to 10 mgm. of the alkaloid per kilo of weight was administered within a period of less than four hours on the last day of the experiment, indicating an extraordinarily rapid rate of elimination, since this amount is equal to about three times the single fatal dose.

Guinea-pig 6 received a total of 41 mgm. of strychnin, or the equivalent of 130 mgm. per kilo, in a period of twenty-seven hours. The condition of the animal before the sixth dose indicated that the first five would have been survived.

Table 1 shows the tolerance of the cat, dog, and guinea-pig to repeated doses of strychnin. The doses are expressed in milligrams of the alkaloid per kilo of weight and in multiples of the single fatal dose; the duration refers to the interval between the first and last doses, and is expressed in hours, disregarding minutes. The rate of elimination is expressed in percentages of the single fatal dose eliminated hourly, and is calculated by subtracting the equivalent of a single fatal dose from the total amount administered (multiple of single fatal doses) and dividing the remainder by the number of hours of the duration.

All doses were administered subcutaneously or intramuscularly except those to cats 10, 11, and 12, and guinea-pigs 6 and 7, which received them orally.

Dog P had been previously poisoned with phosphorus, dogs C–1 and C–2 with chloroform.

Cats are somewhat more susceptible to the toxic action of subcutaneous injections of strychnin than dogs, and the rate at which they eliminate it is less, if calculated in milligrams per kilo of weight, but if calculated in terms of percentage of the single fatal dose there is no great difference when short periods are taken, but the cat does not recover completely so promptly, and does not stand repeated large subcutaneous doses on successive days, so well as the dog. The fatal dose of strychnin for the cat by oral administration often approaches that by subcutaneous injection, owing to the rapidity with which it is absorbed.

TABLE 1

Tolerance of cat, dog, and guinea-pig to repeated doses of strychnin

ANIMAL	NUMBER	DOSE	MULTIPLE OF FATAL	ELIMI-NATION	DURATION	REMARKS
cat		mgm.				
A	1	0.25	1.0			Fatal in 11 minutes
B	1	0.25	1.0			Fatal in 12 minutes
C	1	0.2	1.0			Fatal in 2 hours
1	5	0.7	3.0	25	8.0	
1	10	1.3	5.0	12	32.0	Recovered
2	5	0.7	3.0	25	8.0	Died
3	2	0.35	1.4		2.0	Died
4	5	0.65	2.5	20	8.0	
4	10	1.25	5.0	12	32.0	Recovered
5	4	0.55	2.0		5.0	Died
6	4	0.5	2.0		5.0	Died
7	3	0.4	1.6		4.0	Died
8	4	0.5	2.0	12	8.0	Recovered
9	5	0.6	2.5	18	8.0	Recovered
10	15	3.0	8.0	9	76.0	Recovered
11	36	6.7	20.0	10	244.0	Recovered
12	46	9.0	25.0	8	294.0	Recovered
dog						
A	7	0.8	2.0	14	7.0	
A	13	2.1	5.0		97.0	Recovered
B	8	0.8	2.0	14	7.0	
B	14	2.1	5.0		97.0	Recovered
C	7	0.8	2.0	14	7.0	
C	12	1.8	4.5		97.0	Recovered
D	6	0.86	2.2	17	7.0	
D	11	2.0	5.0		97.0	Recovered
E	1	0.5	1.0			Fatal in 51 minutes
F	1	0.5	1.0			Fatal in 53 minutes
P	9	1.0	2.0	14	7.0	Recovered
C-1	5	0.6	1.0		7.0	
C-1	10	1.2	2.5	5	32.0	Recovered
C-2	5	0.5	1.0		7.0	Died
guinea-pig						
1	12	15.5	4.0	47	6.3	
1	36	52.0	15.0	18	77.0	Died
2	7	16.6	4.4	50	6.3	
2		50.0	14.0	18	75.0	Recovered
4	12	25.0	7.0	55	11.0	Died
5	8	18.0	5.0	57	7.0	
5	18	37.0	10.0	29	31.0	Recovered
6	2	50.0	1.5		2.0	
6	6	130.0	4.0		27.0	Died
7	5	80.0	2.3		5.7	Recovered

The subcutaneous injection of a single dose of 0.4 mgm. of strychnin per kilo of weight, in 1000 parts of normal salt solution, is almost invariably fatal to the dog, but dog D survived the administration of a total of 0.86 mgm. in six doses within a period of seven hours. It is especially noteworthy that this animal survived the injection of a total of 0.4 mgm. per kilo of weight in four doses within six hours after having had a nearly fatal dose, as shown by the severity of the convulsions, and was in a much better condition at the end of this period than at the beginning. Two other dogs, E and F, of the same litter succumbed to doses of 0.4 mgm. per kilo administered in the same way.

Dog D must have disposed of strychnin at approximately the rate of 15 per cent of a single fatal dose hourly. When the injections were made on several successive days and the hourly rate of elimination is calculated for the entire period between the first and last doses the apparent rate of elimination is much less than that shown for the shorter period.

In every case the equivalent of a single fatal dose is subtracted from the total (multiple of single fatal dose) before calculating the rate of elimination because one can never be sure that much less than the single fatal dose is present in the body at the time the last dose is given. Any error which results in this calculation is therefore on the side of conservatism.

The fatal dose of strychnin for the guinea-pig by subcutaneous injection is approximately 3.5 mgm. per kilo of weight, but guinea-pig 1 took a total of more than 50 mgm. per kilo in a period of seventy-seven hours, and while this animal succumbed to the last dose, its condition indicated that it would have survived had not this last dose been given. This view is supported by the fact that a nearly similar amount administered within seventy-three hours to guinea-pig 2 did not prove fatal, and this animal withstood total amounts of 13, 14, and 16 mgm. per kilogram, respectively in three periods of about six hours each, or an amount equal to a single fatal dose every hour and a half.

The fatal dose of strychnin for the guinea-pig by oral administration is about ten times that by subcutaneous injection, though

absorption *begins* fairly promptly. However, the total amounts that can be administered by the mouth over prolonged periods are probably not correspondingly greater than those that can be administered subcutaneously in similar periods of time, thus, guinea-pig 6 succumbed to repeated oral doses amounting to a total of 130 mgm. per kilo in a period of twenty-seven hours, though guinea-pig 7 survived the oral administration of an amount corresponding to 80 mgm. per kilo in a period of five hours and forty minutes.

SUMMARY OF EXPERIMENTS ON TOLERANCE

Total amounts of strychnin equal to several times the single fatal dose may be administered to cats, dogs, and guinea-pigs in fractional doses on each of several successive days, without causing permanent injury, and we must suppose that the poison is eliminated, insofar as its presence in an active state is concerned, as rapidly as it is given in those cases. The mechanism of the elimination, be it by fixation in certain tissues, excretion, or decomposition, remains to be considered.

The guinea-pig eliminates the poison far more rapidly than the cat and dog.

II. EXCRETION

Having shown that the animal body is capable of eliminating strychnin fairly rapidly, we next undertook to determine whether the whole amount administered is excreted unchanged in the urine and feces or mainly decomposed in the body, and the approximate rate at which any of these processes may occur.

We may speak of elimination by storage in the tissues in an innocuous form as a matter of convenience, for the well-being of the animal is served quite as well when the poison is so stored as when it is decomposed or excreted, but even that stored in this way must ultimately undergo decomposition or excretion.

Hatcher and Smith (1) have shown that only very small amounts of strychnin are excreted unchanged in the urine of the dog, except during excessive diuresis, within periods of five hours

following the administration of large doses, but they did not attempt to determine whether a part of the poison is fixed in the body and excreted over prolonged periods. We have therefore examined the urine of cats, dogs, and guinea-pigs for periods of several days, or until even traces of the poison could no longer be detected, following the administration of repeated doses of strychnin, and have also examined the feces of the cat, dog and guinea-pig following the oral administration of very large amounts. It was also sought to determine whether strychnin could be found in various tissues either immediately after the administration of large doses, or at such time as we had reason to believe that elimination (in the sense previously defined) was complete.

While it is convenient to discuss our results under the several headings, we actually used the same animal for studies in tolerance, fixation in the tissues, and excretion, in some cases, hence the experiments lack uniformity to some degree, and this renders tabulation inconvenient.

The following protocols illustrate the results of these experiments.

Experiments on excretion of strychnin in the urine and feces of the cat, dog, and guinea-pig

Cat 12; male; weight 2.94 kilos; excretion in urine.

A total of 26.6 mgm., the equivalent of twenty-five times the single fatal dose, of strychnin was administered orally in a period of a little over twelve days (for details of administration see protocol under tolerance).

The urine, measuring 1520 cc., collected during the administration of the strychnin and for the forty-four hours following was preserved by the addition of chloroform. The first portion, measuring 1000 cc., was evaporated to a small volume which was added to the remainder; the whole was then made alkaline and shaken repeatedly with chloroform; the chloroformic extract was distilled and the residue taken up in 10 cc. of slightly acidulated salt solution. This was tested as follows:

Cat weight 2.5 kilos.

2.46 p.m.   0.5 cc. of extract of urine of cat 12 per kg. intramuscularly.

3.20 p.m.   1.0 cc. of extract as before.

4.00 p.m.   Increased reflex excitability.

4.22 p.m.   1.0 cc. of extract as before.

5.00 p.m.   Reflex excitability markedly increased.   No further effects observed.

Cat 11; female; weight 2.54 kilos; excretion in feces.

A total of 17 mgm., the equivalent of twenty times the single fatal dose, of strychnine was administered orally in a period of ten days.

The feces were collected during the period of administration and for forty-four hours following; rubbed with alcohol; the alcohol evaporated and the residue taken up in dilute acid.   This was then rendered alkaline and shaken repeatedly with chloroform; the chloroform was distilled and the residue was taken up in 5 cc. of acidulated normal salt solution.   The extract was tested by injecting all of it intramuscularly into a cat weighing 2.23 kilos without producing any perceptible effect.

Dog S-4, weight 5.14 kilos; excretion in urine and feces.

A total of 24.16 mgm. of strychnin, the equivalent of about ten times the single fatal dose, was administered orally in a period of six days.

The urine of the last two days of administration and for the four days following, measuring 1700 cc., was collected and extracted in the manner previously described.   The extract, made up to 10 cc., was tested on cats, the result indicating the presence of about 1.5 mgm. of strychnin in the total urine.   The urine of the next sixteen hours, measuring 110 cc., was collected and extracted without evaporation; the extract was tested on a frog with negative results though the presence of as little as 0.05 mgm. would have been detected.

The feces were collected during the administration and for two days following (a small amount lost during two days of diarrhea) and extracted in the manner already described.   The extract, measuring 10 cc., was tested by intramuscular injection into cats; a dose of 2.5 cc.—one-fourth of the whole—per kilo caused no perceptible effect.

Guinea-pig; male; weight 416 grams; excretion in urine and feces.

September 11.   9.50 a.m.   20 mgm. strychnin per kilo, orally.
             10.50 a.m.   10 mgm. strychnin per kilo, orally.
             11.50 a.m.   20 mgm. strychnin per kilo, orally.

September 11.   1.30 p.m.   20 mgm. strychnin per kilo, orally.

            2.30 p.m.   Convulsions, prompt recovery.

            4.50 p.m.   Extreme hyperexcitability.

September 12.   9.30 a.m.   Appears to be normal; chloroformed, gas-
                    tro-intestinal tract, weighing 71 grams,
                    removed.

The urine, measuring 55 cc., and the feces· were collected during the administration of strychnin and until death by chloroform, the feces being placed in alcohol as collected from time to time. The urine, feces, and gastro-intestinal tract with contents were severally extracted, the extract of the feces being made up to 3.5 cc., that of the urine and that of the gastro-intestinal tract and contents to 5.0 cc. each. These were tested on cats, all injections being made intramuscularly, as follows:

Extract of urine; cat, weight 1.93 kilos.

1.10 p.m.   2.5 cc. of extract injected intramuscularly.

1.27 p.m.   2.5 cc. of 'extract (all of remainder) injected intra-
            muscularly.

1.35 p.m.   Hyperexcitable.

1.55 p.m.   Convulsion.

3.45 p.m.   Nearly normal.

Extract of gastro-intestinal tract; cat; female; weight 2.7 kilos.

1.35 p.m.   1 cc. of extract intramuscularly.

1.55 p.m.   4 cc. of extract intramuscularly (all of remainder). No
            certain effect; possibly slight hyperexcitability.

Extract of voided feces; cat; female; weight 2.05 kilos.

11.11 a.m.   2 cc. of extract intramuscularly.

11.45 a.m.   1.5 cc. of extract intramuscularly (all of remainder). No
            perceptible effect.

The following experiments relating to the excretion of strychnin do not require detailed protocols. Cats 1, 4, 8, and 9 received a total of 3.6 mgm. of strychnin in a period of thirty-two hours or less (see table 1). The urines collected during the first four days of the experiment were discarded, after which 120 cc. were obtained from the four cats in the succeeding sixteen hours. This urine was extracted without evaporation, and the extract made up to 5 cc. One cubic centimeter of this extract was injected into the lymph sac of each of two frogs, weighing 20 and

35 grams, respectively, without causing any perceptible effect. Since as little as 0.05 mgm. of strychnin would have been detected by the frog test, we may say that its elimination had ceased within four days after the administration was stopped.

Guinea-pig 8 received a total of 21 mgm. of strychnin by the mouth within a period of twenty-four hours. The urine collected during the administration and the following day, measuring 85 cc., was made up to 100 cc. by the addition of normal salt solution, filtered, and tested by intravenous injection under local anesthesia on a cat weighing 2.6 kilos. The entire amount caused only marked hyperexcitability, indicating the presence of not more than about 0.25 mgm. of strychnin in the urine.

The urine of this guinea-pig was then collected during the next four days and extracted in the usual way. The extract was tested on a frog weighing 35 grams without causing perceptible effect, showing that no strychnin was excreted in the urine of the guinea-pig after the first day following the administration.

Table 2 shows the excretion of strychnin in the urine of the cat and guinea-pig. Those experiments which are not tabulated conveniently, including that on dog S–4, are omitted.

TABLE 2

The excretion of strychnin in the urine of cats and guinea-pigs

ANIMAL	AMOUNT IN MILLIGRAMS		DURATION IN HOURS		VOLUME OF URINE IN CUBIC CENTIMETERS
	Given	Recovered	Administration	Collection	
cat					
10	9.1	0.3	76.0	120	540
11	17.0	0.75	244.0	286	1,000
12	26.6	1.5	294.0	332	1,520
guinea-pig					
2	27.0	1.0	72.0	75	
4	9.0	trace	11.3	12	
5	15.2	0.2	48.0	75	50
7	33.3	0.4	5.6	24	55
8	21.0	0.5	24.0	46	85

Only a small percentage of repeated toxic doses of strychnin can be recovered from the urine of cats, dogs, or guinea-pigs, and none can be recovered after twenty-four to ninety-six hours following the stoppage of administration.

No strychnin can be recovered from the feces of cats, dogs, or guinea-pigs after the administration of such toxic doses.

It is obvious, therefore, that much the larger part of the poison so administered must be destroyed in the body eventually.

III. DESTRUCTION OF STRYCHNIN IN THE BODY

The results already recorded point conclusively to the destruction of strychnin in the animal body, and since total instantaneous destruction of such an alkaloid as strychnin is hardly to be expected, it seemed probable that it is stored in some of the tissues or organs in which it is destroyed, or which give it up slowly to be decomposed.

It is more difficult as a rule to estimate the amount of strychnin present in tissues than that in urine, and the results are often disappointing and difficult to explain satisfactorily. We are far from being the only ones who have experienced such difficulties, however, and not a little of the confusion concerning the recovery of this poison from tissues, with which the literature abounds, is due to the fact that strictly uniform results cannot always be obtained by chemical means, especially where the conditions of the experiment vary.[1]

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[1] Among the many investigators who have reported such difficulties in the extraction of strychnin we may mention Taylor in connection with the celebrated Palmer trial, so frequently cited in the literature. Taylor, himself a chemist, calls attention in the most forcible way to the dangers of drawing conclusions from the failure to recover strychnin from tissues by chemical means. He says "A quarter of a grain of strychnin diffused through 2 or 3 ounces of fluid in a dead stomach may admit of detection, supposing any of the poison to remain there at the time of death; but when spread over or diffused through six or eight pounds of animal matter, including blood and feces, it is an insult to commbn sense to assert that the analysis is not rendered infinitely more difficult and far less certain in its results." Taylor, Alfred Swaine, Poisoning by Strychnin, London, 1856, p. 16.

In the experiments reported in the first two sections of this paper errors of 10, or even 25, per cent in the estimation of strychnin are of no importance so far as the essential facts are concerned, for the difference of a fraction of a milligram in the amount of strychnin excreted in the urine is without practical significance when dealing with doses of 20 to 40 mgm., but such percentage errors are of greater importance in some of the experiments to be reported in the present section. However, we have taken the utmost care to guard against loss in extraction and have made numerous chemical and biological tests of residues after various steps. In some of the earlier control experiments the losses in extraction were so great as to make us doubt the availability of the method, but in the later controls we were able to recover practically all of the strychnin added to large amounts of blood, and with all of the recognized disadvantages, a careful examination of the protocols and tabulated results will show that the essential facts are demonstrated conclusively.

Nearly every investigator who has sought to recover strychnin from animal tissues has found it necessary to modify the methods commonly employed in order to meet varying conditions. We have modified the method in minor particulars and have found the following to give the most accurate results in the extraction of strychnin from blood and tissues.

The blood or tissue is mixed with sand and heated on a water bath with frequent stirring until dry. It is then powdered in a mortar, and alcohol, acidulated with tartaric acid, is added to the powder which is then heated on a water bath with frequent shaking; the mixture is filtered and the residue washed with alcohol. A little acidulated water is then added to the blood or tissue and the mixture is heated on a water bath; a large excess of alcohol is added and the mixture allowed to stand for some hours; this is filtered and the residue washed with alcohol. The mixed filtrates and washings are evaporated or distilled, the residue taken up in dilute hydrochloric acid and filtered; the filtrate with washings placed in a separatory funnel, made strongly alkaline with sodium hydrate and shaken with several successive portions of chloroform; the chloroform is distilled and the residue taken up in slightly acidulated normal salt solution.

Frequently the extract so prepared is clear and almost colorless, but sometimes it requires purification, especially if a gravimetric determination of the strychnin is also to be made.   Control experiments on cats made with extracts prepared by evaporating the alcoholic extract of the tissues containing strychnin and treating the residue with dilute acid, show that the presence of a small amount of organic matter does not greatly influence the accuracy of the biologic test.

Attempts were made to extract the entire tissues of a cat, exclusive of the skin and gastro-intestinal tract, but the process was so time-consuming that the effort was abandoned.

A control experiment on the guinea-pig showed that strychnin could be recovered from the tissues when the extraction was begun immediately after the intravenous injection of an amount very much less than those that were commonly used in the experiments to determine whether storage occurred.

Experiments to recover strychnin from the body of the guinea-pig

A solution of 2 mgm. of strychnin in 1 cc. of normal salt solution was injected into the jugular vein.   Convulsions resulted immediately and death followed in about fifteen seconds.

The skin, liver, and intestines were removed, the remaining tissues weighing 137 grams, were hashed in a meat chopper and extracted. The extract was filtered and the filtrate made up to a volume of 22 cc. and tested as follows: A dose of 0.2 cc., or 1 per cent of the total extract, was injected into the lymph sac of a frog weighing 23 grams and caused lasting tetanus; the intravenous injection of half of the balance of the extract into a cat weighing 1.6 kilos caused nearly fatal convulsions.

The test on the frog indicated the recovery of about three-fourths of the strychnin injected, that on the cat gave a slightly lower result.

Guinea-pig 2, which had previously received a total of 27 mgm. grams of strychnin in three days, was chloroformed three hours after the last dose, the skin and intestinal tract removed; the remaining tissues, weighing 277 grams, extracted and the extract tested biologically; the entire amount after concentration being administered orally to a cat weighing 1.9 kilos without causing perceptible effects. A second cat ate about half of the extracted tissues and exhibited no symptoms.

The tissues, minus skin and intestines, of guinea-pigs 3, 4, 5, 6, and 7 were also extracted.

The total amounts of strychnin administered to these animals, the interval between the last dose and death, and the amount of strychnin recovered from the tissues are shown in table 3.

TABLE 3

Recovery of strychnin from the tissues of the guinea-pig

| ANIMAL | TOTAL DOSE | DEATH | | STRYCHNIN RECOVERED |
		Hours after last dose	Cause	
	mgm.			*mgm.*
3	3	3.0	Chloroform	0.0
4	9	0.6	Strychnin	0.0
5	15	3.0	Chloroform	0.0
6	41	0.5	Strychnin	1.0
7	33	18.0	Chloroform	0.0

The results of the tabulated experiments leave not the least doubt that strychnin is destroyed rapidly in the body of the guinea-pig.

The striking difference between the amounts of strychnin required by oral and subcutaneous administration to cause death in the guinea-pig suggested that a part of the poison might escape absorption into the circulation in this animal and be decomposed in the intestinal tract, and although it had been shown (2) that small amounts of strychnin placed in the cecum of the rabbit were not totally decomposed, we decided to investigate the question of its destruction in the intestinal tract.

The protocol of one of these experiments will serve to illustrate how slight, if any, is this destruction.

Destruction of strychnin by the intestine and contents

Guinea-pig killed by medulla stroke, the intestine excised aseptically, ground with sand and divided into three portions: A, B, and C.

To A added 4.5 mgm. of strychnin in water; mixed and extracted at once as a control.

To B added the same amount of strychnin as in A and placed in incubator for one hour.

To C added strychnin and treated as in B except that it was allowed to stand in incubator for twenty-four hours.

All extracts were made up to 10 cc. each and tested on cats by intramuscular injection. The results were essentially similar in every case, the fatal dose of each extract being 1 cc. per kilo of cat.

The intestinal contents of two guinea-pigs that had received large doses of strychnin by the mouth—guinea-pigs 6 and 7, and four that had had repeated toxic doses by subcutaneous injection, guinea-pigs 2, 3, 4, and 5 were also examined. About 5 mgm. were found in the gastro-intestinal tract of guinea-pig 6, that died within half an hour after the last dose of 6.3 mgm. and a total of 41 mgm. in twenty-seven hours. The intestine and contents of guinea-pig 3, which was killed three hours after the last dose and a total of 3 mgm. in three hours, yielded about 0.3 mgm., in none of the others in which the animal survived the last dose for as much as two hours was more than a very small fraction of the last dose found in the gastro-intestinal tract, but the results show conclusively that at least a small part of the strychnin injected subcutaneously is excreted into the alimentary tract, where it is destroyed or from which it is again absorbed and then destroyed. That the small amounts of strychnin found in the gastro-intestinal tract were not simply held in the blood is shown by the fact previously stated that in only one instance did we find any strychnin in the remainder of the animal body, and in that case the animal, No. 6, died very shortly after the oral administration of the relatively enormous dose of more than 6 mgm.

Since not more than very small amounts of strychnin are destroyed in the intestine of the guinea-pig during a period of twenty-four hours, while very large amounts are destroyed completely in the body of this animal, it is obvious that the intestinal tract is not the principal seat of the destruction.

The certainty that strychnin is destroyed in the body and the possibility that the liver is the organ concerned in its decompo-

sition caused us to investigate the question by means of perfusion experiments on 16 dogs and 2 guinea-pigs.

The dogs were anesthetized and exsanguinated, in most cases while Locke's solution was being injected into the femoral vein in order to facilitate the exsanguination, and the blood defibrinated. The liver was excised, washed free of blood by passing Locke's solution through a cannula in the portal vein, transferred to a perfusion chamber in which the temperature was maintained at 37°C., and perfused with the defibrinated blood to which a weighed amount of strychnin had been added. The perfusion was continued for periods varying from two to six hours, during which time the perfusion fluid usually passed through the liver from twenty to thirty times.

Immediately after the perfusion was stopped the perfused fluid, and usually the liver, were extracted, the extracts, and very frequently many, or all, of the residues were tested biologically on the cat. In a few of the experiments the perfused fluid was tested directly on dogs by intravenous injection, a series of three control experiments having shown that an average of 0.24 mgm. of strychnin per kilo of weight is required to cause death in the dog when pure strychnin is added to 10,000 parts of defibrinated blood and injected in this manner.

One of the perfusion experiments will be detailed.

Perfusion of dog's liver with blood containing strychnin

A dog weighing 10.4 kilos was exsanguinated while 100 cc. of Locke's solution were being injected into the femoral vein. The blood, which after defibrination measured 500 cc., was divided into two portions, 100 cc. being used for a control, and 400 cc. for perfusing the liver. Locke's solution was passed through a cannula in the portal vein to remove the blood from the liver, which was then transferred to a perfusion apparatus in which the temperature was maintained at 37°C., and perfused with the 400 cc. of blood, to which 100 mgm. of strychnin had been added. The blood was shaken with oxygen until bright red after every passage through the liver.

After perfusing the liver for four hours the fluid obtained measured 350 cc., some having been retained in the liver. The liver was then

washed by perfusing it once with Locke's solution, the washings thus obtained measured 1075 cc. and will be referred to hereafter as the "washings." The liver then weighed 411 grams or a little more than the perfused fluid and about two-fifths as much as the "washings."

Twenty-five mgm. of strychnin were added to the 100 cc. of defibrinated blood used as a control, and this was extracted at once in the manner that has been described. The perfused fluid, the "washings," and the liver were also similarly extracted, and the extracts in every case made up to 25 cc. The strychnin content of each was estimated by means of the biologic test, after which an aliquot part of each was taken and the total strychnin content of the mixture was estimated in the same way. The protocol of the tests follows. All injections were made intramuscularly in the cat.

Extract of the perfused fluid; 25 cc. total

CAT	DOSE CUBIC CENTIMETER PER KILOGRAM	RESULT
1	0.5	Hyperexcitability only, nearly normal in 2 hours
2	1.0	Moderate tetanus in 11 minutes, recovery
3	1.2	Extreme hyperexcitability, recovery

Extract of "Washings;" 25 cc. total

CAT	DOSE CUBIC CENTIMETER PER KILOGRAM	RESULT
1	0.35	Hyperexcitability only
2	0.45	Moderate tetanus, once repeated, recovery
3	0.60	Tetanus and death in 5 minutes

Extract of liver; 25 cc. total

CAT	DOSE CUBIC CENTIMETER PER KILOGRAM	RESULT
1	0.5	Tetanus in 8 minutes, recovery
2	0.6	Tetanus in 13 minutes, recovery

Extract of control blood; 25 cc. total

CAT	DOSE CUBIC CENTIMETER PER KILOGRAM	RESULT
1	0.35	Tetanus in 16 minutes, and death
2	0.50	Tetanus in 10 minutes, and death

Mixed aliquot parts

CAT	DOSE CUBIC CENTIMETER PER KILOGRAM	RESULT
1	0.30	Slight tetanus, recovery
2	0.40	Slight tetanus, once repeated, recovery
3	0.50	Severe tetanus, nearly fatal
4	0.60	Severe tetanus in 10 minutes and death

Summary of tests

EXTRACT	FATAL DOSE IN CUBIC CENTIMETERS	STRYCHNIN RECOVERED
		mgm.
Perfused fluid................................	1.2	6
"Washings"................................	0.5	15
Liver....................................	0.75	10
Control...................................	0.3	25
Total....................................		56
Aliquot parts...............................	0.55	55
		in 100 cc.

While death did not occur in any of the tests of the extract of the perfused fluid or that of the liver, the severity of the symptoms showed that the fatal dose must lie very near to the highest used in these cases. In the test of the "washings" the largest dose was so promptly fatal, and the next largest so nearly fatal that the fatal dose is placed nearer the lower of these two. The more exact test of the mixed aliquot parts of all four of the extracts proves that any error in the figures of the individual tests is without significance so far as the main facts are concerned.

The residues of the liver, perfused fluid, and "washings" left after the extraction were again extracted with acidulated dilute alcohol, the alcohol evaporated, and the residue tested biologically on cats. The result indicated that about 1 or 2 mgm. of strychnin remained in the residues after the first extraction, and this amount added to that indicated above shows that 58 mgm. of strychnin were recovered. Of this 25 mgm., the amount originally added, were obtained from the control blood, hence only about 33 mgm. remained in the liver, the perfused fluid, and the "washings," after perfusion, indicating conclusively that much the larger part of that added to the perfused fluid was destroyed during the perfusion of the liver.

It is to be observed particularly that the perfused fluid, measuring 350 cc., contained only about 6 mgm. of strychnin, while the liver, which weighed 411 grams, or about 22 per cent more than the perfused fluid, contained, before washing with the liter of Locke's solution, about 25 mgm., or four times as much

as the perfused fluid. There can be no question, therefore, that the liver actually stores strychnin during its perfusion, and since the amounts present in the perfused fluid and "washings" were nearly proportional to their volumes, it is obvious that the strychnin is only loosely bound in the liver, probably being distributed according to its relative solubility in the liver fluids and those used in perfusion, including the blood.

The fact that strychnin is destroyed during its perfusion of the liver with blood does not prove that the liver alone is concerned in the destruction, and it is conceivable that the blood might share in this action, or even might be the sole agent of destruction, as some have maintained. We attempted therefore to throw some light on the mechanism of the destruction by means of three types of experiment: (1) Maceration of a mixture of hashed liver tissue with strychnin solution at body temperature. (2) Maceration of blood with strychnin at body temperature. (3) Perfusion of the liver with Locke's solution, instead of blood, containing strychnin.

The control test of the recovery of strychnin immediately after its addition to defibrinated blood, shows that it can be recovered practically quantitatively, and the following experiment was performed to determine whether blood decomposes strychnin.

Behavior of blood toward strychnin

A dog was exsanguinated and 320 cc. of defibrinated blood were obtained under aseptic conditions; 110 mgm. of strychnin were added and the blood placed in an incubator where it remained for forty-eight hours at body temperature. At the end of this period it was divided into two portions, the first portion, measuring 29.2 cc., and containing 10 mgm. of strychnin (supposing none to have been destroyed) was diluted with an equal volume of normal salt solution and tested directly on dogs by intravenous injection. The second portion, containing 100 mgm. of strychnin (supposing none to have been destroyed) was extracted, the final extract being made up to 100 cc., and tested on cats by intramuscular injection. The results of the tests are given below, but for convenience the doses of the diluted blood are expressed in terms of the undiluted.

Direct test of incubated blood

	FATAL DOSE	REMARKS
dog	*cc. per kgm.*	
1	0.60	Injection too rapid, hence dose slightly too large
2	0.46	Tetanus after 0.27 cc. per kilogram
3	0.40	Tetanus after 0.28 cc. per kilogram

Test of extract of incubated blood

cat		
1	0.25	Tetanus in 9 minutes; recovery
2	0.30	Tetanus in 11 minutes and death
3	0.35	Severe tetanus in 14 minutes; recovery

The average fatal dose in the three direct tests on the dog was 0.47 cc. of the blood per kilo, hence 29.2 cc. would have been fatal to 62 kilos. This was a greater degree of activity than shown in the tests in which strychnin was added to 10,000 parts of blood and tested at once, but in this case the dilution was only 1 to 5840.

The results of the tests of the extract on cats 1 and 2 were in close agreement, the fatal dose being 0.3 cc. per kilo and, showing that 100 cc. of the extract would have been fatal to 333 kilos of cat indicating that 100 mgm. had been recovered from the second portion of the blood, hence that no destruction had occurred. We are not prepared to say that the blood never destroys small amounts of strychnin, on the contrary, some of our results seem to point in that direction, but they would not explain the destruction that occurs during perfusion of the liver.

Behavior of liver tissue toward strychnin during maceration

A dog weighing 6.9 kilos was chloroformed, the liver was excised, hashed in a meat chopper and divided into two portions, A and B. One hundred mgm. of strychnin were added to each portion; A was extracted at once, B after it had been allowed to remain for two hours at body temperature. The biologic tests on cats showed that

practically all of the strychnin added to each portion had been recovered in the extracts, the results of the tests on the two portions being in striking agreement.

The results of previous experiments in which perfused livers were·permitted to remain at body temperature for a time before extraction seemed to point in the same direction, but they were not conclusive.

Behavior of liver toward strychnin during perfusion with Locke's solution

A perfusion experiment was conducted in the manner already described, except that Locke's solution was used in place of the defibrinated blood. To this 100 mgm. of strychnin were added. The perfused fluid measured 450 cc., the liver weighed 225 grams, being just half the weight (or volume) of the perfused fluid, but at the end of the perfusion the liver contained the same amount of strychnin as the perfused fluid—37 mgm.—and of this 30 mgm. were removed by the perfusion of 1000 cc. of fresh Locke's solution once through the organ.

The distribution of the strychnin between liver and perfused fluid in this case (experiment 12) was similar to that seen when defibrinated blood was used, the liver storing about twice as much as would be present if the alkaloid were evenly distributed through liver and perfused fluid, but in experiment 11, in which Locke's solution was also used, there was no apparent storage in the liver, the distribution being uniform through liver and perfused fluid.

It is interesting to know that the destruction of strychnin was less active in this experiment than in the greater number of those in which defibrinated blood was used, but we are unable to say whether this is to be explained by the difference in the perfusion fluid or by the peculiarity of the individual liver, since the destruction occurred at widely different rates in the several experiments. It is more than possible that the blood participates in the destruction during perfusion, even though it does not appear to possess the power to destroy the alkaloid unaided.

At this stage of our investigation Richard Weil called our attention to an alteration in the behavior of the liver of the

dog toward certain dyes during phosphorus poisoning, and it seemed possible that there might be an analogous change in its capacity for storing and destroying strychnin.

Perfusions were carried out on the livers of two dogs that were nearly moribund after repeated doses of phosphorized oil.   These livers presented the appearance that is typical of phsophorus poisoning, with droplets of fat scattered freely through their substance.

The well known toxic action of chloroform on the liver lead us to employ the liver of a dog suffering from chronic chloroform poisoning for a similar experiment.

The results of these three experiments, in harmony with those on tolerance of such poisoned animals toward repeated subcutaneous injections of toxic doses, indicate that there is no essential difference in the capacity of the normal liver and those of dogs poisoned with phosphorus or chloroform to destroy strychnin, though it is possible that there is some impairment of the function in such cases.

EXCRETION OF STRYCHNIN IN THE BILE

While the results of the experiments already reported left no doubt in our minds that strychnin is decomposed during the perfusion of the liver, it seemed to us that the intact animal might excrete at least a portion of that which is stored in the liver, and this seemed the more probable in view of the fact that at least traces of the poison were found in the intestine after its subcutaneous injection into the guinea-pig.   The following experiment was performed in order to decide the question.

A dog was anesthetized and cannulas were inserted, one into the femoral vein and one into the gall gladder after the gall duct had been tied.   The animal was kept fully under the influence of hydrated chloral, permitting of the intravenous injection of very large doses of strychnin.   This was injected with frequent interruptions, during which convulsions sometimes occurred and which required an additional dose of hydrated chloral for their suppression.   After a total of 5 mgm. of strychnin had been

injected the animal was allowed to remain for two hours, during which time a total of 75 cc. of bile was collected. This was extracted, and the extract tested on frogs but not a trace of strychnin could be found though as little as 0.05 mgm. in the bile would have been detected.

The protocol of this experiment is given in brief.

Excretion of strychnin in the bile of the dog

Female; weight 9.12 kilos; ether anesthesia, insertion of cannula into the gall bladder after tying duct, and cannula into the femoral vein for injection from burette.

10.15 a.m.   0.3 gram hydrated chloral per kilogram by rectum.
10.30 a.m.   Began injection of strychnin solution 1–10,000.
12.17 p.m.   0.2 gram hydrated chloral per kilogram by rectum.
12.47 p.m.   5.0 mgm. strychnin total injected.
 2.40 p.m.   Violent tetanus; artificial respiration.
 2.50 p.m.   Heart stopped.

The bile, measuring 75 cc., secreted during the experiment was rendered strongly alkaline by the addition of sodium hydrate solution and shaken with several successive portions of chloroform; the chloroform was distilled; the residue taken up in 5 cc. of slightly acidulated normal salt solution. The extract was not bitter and 0.5 cc. of it failed to give any reaction with Mayer's reagent. It was tested on a frog as follows:

Frog, weight 32 grams.

4.03 p.m.   0.5 cc. extract into lymph sac.
4.35 p.m.   1.0 cc. extract into lymph sac.
5.05 p.m.   No definite strychnin action, possibly slight hyperexcita-
            bility, uncertain.
Next morning, normal.

The bile left after extraction with chloroform was acidulated and evaporated nearly to dryness, the residue treated with acidulated alcohol, the alcohol evaporated, the residue taken up in 6 cc. of slightly acidulated normal salt solution. All of this extract was injected intravenously into a cat weighing 2.5 kilos without producing any perceptible effect. Had the extract contained as much as 0.25 mgm. of strychnin it would have produced distinct hyperexcitability in the

cat; as little as 0.015 mgm. would have caused pronounced hyper-excitability in the frog that we used.[2]

The results of all perfusion experiments are tabulated.   Table 4 shows the duration of the perfusion in hours, the weight of the liver, the amount of strychnin added to the perfusion fluid, the

TABLE 4

EXPERIMENT	DURATION IN HOURS	LIVER, WEIGHT IN GRAMS	MILLIGRAMES STRYCHNIN		PERCENTAGE DESTROYED
			Added to fluid	Total recovered	
dog					
1	4	400	15	2	87
2	5	310	10	4	60
3	2	220	30	8	73
4	2	228	100	47	53
5	4	284	100	10	90
6	6	230	100	6	94
7	6	325	100	25	75
8	5	324	100	45	55
9	4	478	50	28	44
10	6	250	50	20	60
11	5	450	20	7	65*
12	4	225	100	74	26*
13	4	411	100	33	67
14	5	190	100	60	40†
15	6	360	100	35	
16	5	287	100	23	77‡
guinea-pig					
1	4	23	20	9	55
2	2		10	3	70

* Locke's solution used in place of defibrinated blood for perfusion.

† Liver of dog previously poisoned with chloroform.

‡ Liver of dog previously posioned with phosphorus; the liver of dog 15 was not extracted, but was perfused with Locke's solution after perfusion with poisoned blood was completed.

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[2] It may appear that we have used excessive caution in guarding against error in the extraction of strychnin to the point of suggesting lack of confidence in our own methods.   The certainty that others have failed to detect strychnin when it was present in tissues in notable amounts, the desire to avoid errors and to convince the most skeptical, thereby putting an end to the confusion that now exists in the literature with reference to the points under discussion, have seemed to us to justify even many other experiments of this nature, which, however, do not require detailed consideration here.

amount recovered, and the amount destroyed during perfusion. Table 5 gives the ratios of the weight of the liver, its strychnin content, and strychnin concentration to those of the perfused fluid. The ratio of strychnin concentration is readily computed from the figures in the first two columns of this table, and is given here merely for the convenience of the reader.

TABLE 5

EXPERI-MENT	WEIGHT		STRYCHNIN CONTENT		STRYCHNIN CONCENTRATION*	
	Liver	Fluid	Liver	Fluid	Liver	Fluid
dog						
3	3 :	10	1 :	1	10 :	3
4	4 :	9	2 :	1	18 :	4
5	1 :	2	3 :	2	3 :	1
6	4 :	9	1 :	1	9 :	4
7	5 :	6	9 :	5	2 :	1
9	8 :	5	7 :	2	2 :	1
10	1 :	1·	1 :	1	1 :	1
11	9 :	8	6 :	5	1 :	1
12	1 :	2	7 :	8	9 :	5
13	8 :	7	4 :	1	7 :	2
14	5 :	9	3 :	2	5 :	2

* In some of the experiments the perfusion of the liver with Locke's following that with the strychnin solution or fluid, prevented the determination of the ratio of concentration, hence their omission from table 5.

DISCUSSION OF TABULATED PERFUSION EXPERIMENTS

There is no observable relation between the duration of an experiment or the weight of the liver and the percentage of strychnin destroyed during perfusion. In some cases this may be explained by the fact that only a part of the liver participated in the perfusion. It would not be profitable to speculate extensively at this time concerning the probable explanation of the wide differences in the capacities of the liver for decomposing strychnin, desirable as such an explanation is, but it is conceivable that the excised liver is incapable of destroying more than a certain amount of strychnin, by means of a hypothetical substance and that the destruction is completed within two hours.

We have already stated that in some of our earlier experiments disturbing errors occurred, at least, we were unable to recover all of the strychnine which we had reason to suppose that we should, but there is no essential difference in the averages of the results obtained in those and the later experiments, and further-more, we made the most extensive tests of residues in some of those in which we suspected errors without being able to detect a trace of strychnin in the previously extracted tissues, residues, or precipitates. Various means were employed in testing these residues. The blood or tissue after extraction was dried and fed to cats, or again extracted with hot acidulated water or dilute alcohol, the extract evaporated, all alcohol being expelled, and the filtered extract administered to cats through a stomach tube. In some cases the several precipitates were extracted in this way and tested, and in others all residues of every description, in-cluding extracted tissues, precipitates, and fluids which had been shaken with chloroform were mixed, heated with acidulated dilute alcohol, the alcohol evaporated, the extract filtered, ren-dered nearly neutral and administered to cats through a stomach tube.

The results of these supplementary tests of impure extracts of tissues, precipitates, and other residues were negative in dog experiments 1, 3, 6, and 15, but this does not mean that traces of strychnin may not have been overlooked. Amounts varying from 1 to 2 mgm. were found in the residues in experiments 2, 5, 7, 12, 13, and 16, and nearly 4 mgm. in experiment 8. The greater part of the last mentioned amount was obtained from the blood residue which had been extracted with strong alcohol with-out the addition of acid, a method of extraction which seemed to hold out certain advantages but which was abandoned because of this imperfect result. Naturally, the amounts of strychnin found in these supplementary extractions are taken into account in estimating the destruction during perfusion. In a few experi-ments these supplementary tests were omitted.

Emesis followed the administration of such crude extracts in some cases, especially when they contained large amounts of inorganic salts; in subsequent experiments these salts were pre-

cipitated from concentrated extracts by the addition of a large excess of alcohol. The precipitated salts were nearly white and were devoid of bitterness. Emesis regularly followed the oral administration of certain extracts of the liver of the guinea-pig, even after purification, and it was evident that that organ contains, at times, at least, an actively emetic substance, and, it may be remarked, parenthetically, that the intestinal tracts of various animals yield actively depressant substances. These were not studied further.

When one reflects that the amount of strychnin commonly used in our experiments, 100 mgm. in most cases, was sufficient to cause marked hyperexcitability in nearly 1000 kilos of cat, it will be understood that the administration of a large part of the residues or impure extracts to a cat without inducing perceptible effects precludes the presence of more than negligible amounts of strychnin.

In a few cases we made gravimetric determinations of the strychnin in the extracts that had been tested biologically. In none of these did we obtain a much larger amount of impure strychnin than was indicated by the biologic tests. The result of such a determination in experiment 12 may be reported briefly, though we are well aware that the extremely close agreement in this case must be attributed to good fortune, and could not be duplicated in every case without very extensive biologic tests. Experiment 12 was almost an exact duplicate of the one that has been detailed except that a much smaller percentage of the strychnin was destroyed. The extracts of the liver, "washings," and perfused fluid were tested separately, after which the remainders were mixed and tested on five cats, the closely concordant results showing that the total of 70 cc. of the three extracts would be fatal to 233 kilos of cat by intramuscular injection, indicating the presence of 70 mgm. of strychnin. Fifty-nine cubic centimeters of the mixed extracts, or 84.3 per cent of the whole, were alkalinized and shaken several times with chloroform; the chloroform was evaporated, leaving a brownish residue weighing 0.0576 gram, corresponding to 0.0683 gram in 70 cc.

The biologic tests indicated the presence of 43.5 mgm. of strychnin in 74.75 cc. of mixed extracts, including part of that of a control, in experiment 13; a chloroformic extract of this amount of the mixed extracts yielded on evaporation 46.2 mgm. of impure strychnin.

DISCUSSION

The problem concerning the behavior of strychnin in the body is of importance with reference to its dosage in therapeutics, especially with reference to variations in dosage dependent on the mode of its administration, and also with regard to the treatment of poisoning by it.

Two questions, concerning which there has been an almost total want of agreement, are that of the excretion of all of the strychnin in the urine unchanged and that of its storage in the liver with the possible destruction of at least a portion of it in the body.

Adam (3) is said to have been the first to detect strychnin in the urine, and it is now known beyond dispute that strychnin begins to appear in the urine of man and animals shortly after its entrance into the circulation, and that traces at least may be found in the urine several days after its administration has ceased in some cases.

It is frequently stated that it is completely excreted unchanged in this way, but a review of the literature affords no satisfactory evidence to support this view, and so far as we know no one has ever succeeded in recovering from the urine as much as 50 per cent of the strychnin ingested, but on the contrary, with so few exceptions that they are almost negligible the amounts recovered have been far less than those administered.

Kratter, 1882 (4) is probably the most frequently quoted in support of the view that all of the ingested strychnin is excreted in the urine unchanged, but there is nothing in Kratter's work to justify his conclusions, which have been generally accepted with very little critical examination. Sollmann (5) states that strychnin is presumably partly destroyed in the body, perhaps mainly in the liver.

J. Dixon Mann, 1889 (6), states that the rapid elimination of strychnin gave rise to the belief that it is decomposed in the body. He also states that it is present in the liver only to the extent that it is held in the blood present in that organ, and that numerous observations on man and experiments on animals prove that it is practically indestructible in the body, and that it is eliminated chiefly by the urine unchanged.

Such dogmatic statements, which can result only from a superficial examination of the literature, and must disregard the work of Cloetta and that of Masing (7) (a pupil of Dragendorff's), have tended to perpetuate the erroneous beliefs now existing regarding the fate of strychnin in the body.

von Rautenfeld, 1884 (8), recovered 6.1 mgm. of strychnin from the urine of two very old people who had taken a total of 16 mgm. in a period of about four days. He detected traces of strychnin on the sixth day after the administration had been stopped, and concluded that there was no foundation for the belief that strychnin was decomposed in the body.

Cloetta, 1866 (9), injected doses of 20, and 25 grains of strychnin into the veins of two horses, death resulting in twenty-six, and thirty, minutes respectively. He was unable to detect a trace of strychnin in the blood, liver, heart, or urine, though he claimed that by his method he could detect one-twentieth of a grain in 650 cc. of urine.

The literature relating to the destruction of strychnin in putrefying tissues is also contradictory, the confusion appearing to have resulted from a failure to distinguish between the partial and total destruction of the alkaloid under the conditions of the experiments.

Numerous investigators have found that traces of strychnin could be found in putrefied tissues in which it had remained for years, and it has been argued from this that strychnin is not at all destroyed during such putrefaction. In fact, the experiments in question demonstrate pretty conclusively that vastly the larger part of the strychnin added had been destroyed. It is not easy to explain just why very large amounts of strychnin are decomposed rapidly during putrefaction and traces resist for

long periods, but possibly it is a question of concentration.   One or two examples of these experiments will suffice for our purpose.

Ranke, 1882 (10), poisoned nineteen dogs with doses of 100 mgm. of strychnin nitrate each, and buried their bodies for periods varying from one hundred to three hundred and thirty days, after which they were examined for strychnin.   In no case—even after the shorter period—was the chemical test conclusive, despite the relatively enormous dose of strychnin employed, hence we must suppose that at the end of one hundred days only traces of strychnin remained, or at least, very small amounts.   After three hundred and thirty days traces of strychnin could be detected by the frog test, and we must suppose that destruction must have been exceedingly slow during the last two hundred and thirty days.

Another experiment that is often cited to prove that strychnin is not decomposed during putrefaction is no more convincing of that view than is that of Ranke's just cited.   Riekher (11) added 5 grains of strychnin to a quarter pound of meat and kept it in a glass jar in an attic for eleven years, after which strychnin could be detected.

The statement that strychnin is not decomposed during putrefaction is a most pernicious one, and it has lead to the claim that the failure to detect strychnin in the exhumed corpse after suspected murder precludes strychnin as the cause of death. It is curious that the detection of traces of the poison in putrefied tissue is used as an argument that none is decomposed, while the detection of traces in the urine is urged as proof that all is excreted by the kidneys.

While many deny that the liver is capable of fixing strychnin the evidence afforded by the literature that strychnin is found more abundantly in the liver than could be accounted for by its presence in solution in the blood contained in that organ is overwhelming.   The arguments to the contrary are mere assumptions, based on the fact that the liver contains a large proportion of the total blood, or they result from the examination of livers from which the strychnin had been removed for the greater part by washing, as in our perfusion experiment already discussed.

That strychnin is not firmly bound in the liver appears to be true, that it is loosely bound, appears to be incontestable.

It is nothing else than astonishing that the question of the partial or complete destruction of strychnin (its change into an innocuous form, constituting destruction) in the body should have remained so long a matter of dispute, considering the extreme simplicity of the experiments required to settle the question definitely. We refer to the continued administration of large doses and the subsequent examination of the excreta and the tissues of the animal.

The chief reason perhaps for the neglect of this question is that investigators have been so strongly impressed with the widespread belief that strychnin resists decomposition even during years of putrefaction, and that it is excreted completely unchanged in the urine, that it did not seem worth while to undertake a time-consuming investigation.

As a matter of fact, Kuenzer, 1914 (12), did actually demonstrate fairly conclusively that strychnin is decomposed in the body of the guinea-pig, but he does not appear to have appreciated the significance of his results, for he says the question of its destruction in the body arises since he never recovered from excreta and tissues more than 2 per cent of that administered though he injected enormous amounts of the insoluble alkaloid subcutaneously, this being followed by extremely slow absorption without symptoms.

de Vámossy, 1904 (13), discussed the literature relating to the fixation of poisons in the liver and concluded as the result of his perfusion of the liver of the rabbit that that organ fixes strychnin with the formation of an insoluble compound of nuclein with the alkaloid. There is nothing in his conclusions that is incompatible with our results, but we have made no effort to confirm them.

SUMMARY AND CONCLUSIONS

Experiments on cats, dogs, and guinea-pigs show that toxic doses of strychnin may be administered at short intervals during periods up to twelve days, the total amounts so administered be-

ing equal to twenty-five times the single fatal dose, without causing perceptible lasting effects.

Only a small percentage of the strychnin so administered can be recovered from the urine, and none from the feces. The excretion in the urine usually ceases within twenty-four to forty-eight hours.

The tissues of the guinea-pig (exclusive of the skin, which was not examined) do not yield any strychnin even after the administration of very large amounts provided that death does not take place within three hours after the administration of the last dose.

The facts just stated point conclusively to the rapid destruction of strychnin in the body of the guinea-pig, and almost as conclusively to that in the bodies of the cat and dog.

Perfusion of the liver of the dog and that of the guinea-pig with defibrinated blood or Locke's solution to which strychnin has been added results in the destruction of a large part of the strychnin, and the storage of a greater portion of the remainder than can be accounted for by the proportion of the perfused fluid held in the liver.

The strychnin stored in the liver is loosely bound and the greater part of it may be removed by perfusing once with an amount of Locke's solution equal to several times the weight of the liver.

Strychnin is not destroyed in all cases when it is added to defibrinated blood or hashed liver tissue and allowed to stand at body temperature for several hours, but there is some evidence that small amounts may be destroyed in this way under slight differences in conditions which we have not determined.

Strychnin is destroyed slowly, or not at all, when it is added to the guinea-pig's intestine and its contents and the mixture is allowed to stand at body temperature for twenty-four hours.

Strychnin is not excreted in the bile after its intravenous injection into the dog.

The biologic test permits of the quantitative estimation of strychnin in tissues far more accurately and conveniently, in many cases, than is possible by chemical means alone.

The confusion and contradictions in the literature are discussed briefly and their causes explained. The statement that strychnin is completely excreted in the urine unchanged is based on assumptions alone, and has no experimental or other evidence to support it. The statement that it is not decomposed during putrefaction is equally an assumption, and is clearly disproved by the very experiments usually cited in support of that view.

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ATTEMPTS TO PRODUCE A SUBSTANCE WITH THYROID-LIKE ACTIVITY BY THE ARTIFICIAL IODIZATION OF PROTEINS

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It has been shown by Morse (1) and confirmed by us (2) that artificially iodized blood serum (Iodalbin, Parke, Davis and Company), when fed to tadpoles, causes acceleration of metamorphosis similar to the effects of feeding with desiccated thyroid, although not as rapidly nor as marked, in comparison with the amounts of iodine present. We have attempted to determine whether such activity can be demonstrated after artificial iodization of the component protein fractions of blood serum obtained by the crude albumen-globulin separation resulting from salting the serum with ammonium sulphate, and also if proteins other than those represented by blood serum showed this activity when iodized. In addition, we have investigated whether such activity can be increased by concentration of the products, by the method of alkaline hydrolysis employed by Kendall (3) in concentrating the active principle of the thyroid.

Employing the method described by Kurajeff (4) we have artificially iodized whole ox serum, ox serum globulin (obtained by half-saturation of the serum with ammonium sulphate), ox serum albumen (obtained by saturating the filtrate of the globulin with ammonium sulphate), Merk's egg albumen and egg white (obtained from fresh eggs). We also used commercial iodized blood protein (Iodalbin) and Iodocasein (Mulford). From all of these iodized proteins we also prepared products "A" (Kendall) by alkaline hydrolysis.

The iodine contents of these preparations are given in table I. The artificially iodized proteins and their products "A" show wide variations in their iodine contents. No particular significance is attached to these differences, since no attempt was made to completely remove the free iodine.

The plan of feeding was the same as described in our previous work, in which the tadpole reaction was employed. All experiments were carried out in duplicate. The tadpoles received liver every other day and a dose of the substance to be tested on the alternating days. The tap water in the dishes was changed twice daily.

Each of the iodized proteins was offered in doses of 1, 2 and 5 mgm. and their products "A" in doses of 0.5, 1 and 2 mgm. Product "A" of fat-free hog thyroid was fed to one set of tadpoles and the activity observed for comparison with the other preparations. This product, as well as the desiccated thyroid from which it was obtained, had been used in our previous investigations and our familiarity with its activity led us to employ it for comparison with these substances.

Two series were studied, one on young tadpoles (started May 28 and about twelve days old) and another on older ones (started June 20 and about twenty-five days old). We have previously pointed out that the most reliable results are obtained with older tadpoles (2 and 5). The results obtained are given in condensed form in table 1. Our chief desire in the present investigations being to ascertain whether any of the preparations demonstrate a distinct action, we have made no attempt to determine their quantitative activities, and the results in the table represent the composite activity of all doses administered. Very marked activity is indicated by I, marked activity II, moderate III, weak IV, very weak V, doubtful VI, and normal growth (as shown by the controls) VII.

A striking difference between the iodized proteins and thyroid was indicated from the results obtained by feeding their products "A" to tadpoles. Alkaline hydrolysis of the thyroid always concentrates the active iodine yielding the very active product "A" while with the iodized proteins it is apparent that

the process of hydrolysis disrupted the active iodine combination, so that the resulting product "A" gave practically no effect. This leads to the conclusion that the iodine complex in thyroid, being in a much more stable combination, is not identical with that of the iodized proteins. The very slight activity, observed in a few instances, of the products "A" can be explained by the fact that the crude process of hydrolysis may have been more or less incomplete with some of the preparations, and the separation of the product "A" from the iodized

TABLE 1

IODIZED PROTEIN	IODINE CONTENT	ACTIVITY SERIES 1	ACTIVITY SERIES 2	IODINE CONTENT OF PRODUCT "A"	ACTIVITY OF PRODUCT "A" SERIES 1	ACTIVITY OF PRODUCT "A" SERIES 2
	per cent			per cent		
Iodized ox serum	11.69	III–IV	II–III	6.15	IV	V–VI
Iodized ox globulin ..	20.3	II–III	I–II	20.3	VI	VI
Iodized ox albumen..	20.3	III–IV	III	17.53	V–VI	VI
Iodalbin (Parke, Davis & Co.)*	21.5	IV	IV	6.15	V	V–VI
Iodocasein (Mulford)	18.0	IV	III–IV	6.76	V	V–VI
Iodized egg albumen (Merk)	12.3	V	IV–V	12.92	VI	V–VI
Iodized fresh egg white.............	11.69	V	III–IV	14.15	VI	V–VI
Fat free hog thyroid.	0.21			1.02	I	I
Controls............		VII	VII		VII	VII

* Activity was distinctly lower than a specimen used in another series of experiments.

protein also being crude, the product may contain a small quantity of the material from which it is derived, which is still unchanged.

The iodized egg protein caused a very little effect, that prepared from fresh egg white being somewhat more active than the preparation made from commercial egg albumen (Merk's).

Casein being an isolated protein, it is of interest to note that when iodized (Iodocasein) it is capable of showing activity on tadpoles. This is in harmony with the accepted view that the active iodine complex of the thyroid is in combination with

tyrosine or tryptophane, casein being relatively rich in both these amino acids (tyrosine 4.5 per cent and tryptophane 1.5 per cent), to which its activity when iodized may be due. In this regard it is interesting to note that egg albumen is relatively poorer in tyrosine (1.1 per cent) than either casein, serum-albumen (2.1 per cent), or serum-globulin (2.5 per cent).

In these experiments, we found Iodalbin to show distinct activity, but much less than another specimen of Iodalbin showed in another set of experiments. This product showed distinctly less activity on the tadpoles than the iodized blood proteins prepared by us, while the specimen used in our other set of experiments was fully as active. This can possibly be explained on the supposition, that different specimens of blood vary in their contents of the necessary nucleus with which the iodine must combine.

It appears from the results obtained by us with the tadpole reaction, that this nucleus is most abundant in the globulin fraction of the blood serum. It will be seen that the greatest activity displayed by any of the artificially iodized proteins was shown by the serum globulin. The albumen fraction was somewhat less active than the whole serum. Allowing for the crudeness of the separation, the indications are that the globulin fraction represents most of the activity represented by iodized blood serum. The fact that different sera when iodized show differences in activities also indicates that the amount of the necessary nucleus present varies in the different bloods.

The striking activity exhibited by the iodized ox serum globulin is a very interesting observation, in as much as the active iodine of the thyroid is known to be in combination with the thyroid globulin. It is indeed possible that in the animal organism, iodine is carried to the thyroid in combination with the serum globulin and is then converted into the thyroid hormone by further action contributed by something (probably an enzyme) in the thyroid. The probability of this hypothesis is strengthened by the fact that no activity can be elicited from hyperplastic thyroids or their concentrated product "A" when artificially iodized (2) and also by the fact that alkaline hydrolysis destroys the activity of iodized serum globulin, therein

differing from the activity of the thyroid, which may indicate that the globulin of the blood contains the necessary nucleus (amino acid groups) with which the iodine must combine, and the thyroid supplies the activating substance (enzyme?) both of which must come into contact with each other to complete the stable iodine combination or hormone which is active in the thyroid and is concentrated by alkaline hydrolysis. More information on this point might be revealed by investigating the activity of iodized sera obtained from goiterous animals and from thyroidectomized animals, which we had hoped to study in the near future, but owing to the fact that one of us (Dr. Marine) has been assigned to military duty, the progress of this investigation has been interrupted and we have deemed it advisable to report the results obtained by us up to the present.

SUMMARY

1. Artificially iodized blood serum (ox) causes acceleration of metamorphosis when fed to tadpoles, resembling that shown by thyroid, but not so marked nor so rapid.

2. It is possible to show by crude process of separation that the globulin fraction of the serum contains most of the substance with which the iodine combines to give the activity on tadpoles.

3. Alkaline hydrolysis of iodized proteins apparently destroys their activity, therein differing from thyroid. This suggests that the thyroid adds something to the iodine complex in the blood to complete the stable iodine containing thyroid hormone.

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THE INFLUENCE OF ERGOTOXIN ON BODY TEMPERATURE

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The enormous literature concerning the physiologic action of ergot is confined almost entirely to the action of the drug on the sympathetic nervous system and the structures innervated by it. In fact most authors limit their attention to its influence on the uterus and on the circulatory system, other effects of the drug being passed over entirely or mentioned merely incidentally. The very marked action of ergot on the body temperature seems to have escaped the attention of pharmacologists.

The earliest mention of any effect on the temperature appears to be in an article by Hemmeter (1), who made a detailed study of the pharmacology of ergotole.[1] He studied the effect on several species of birds and of mammals, including cocks, pigeons, cats, rabbits and man. He saw in all a marked fall of temperature. It is probable that he was working with a preparation which did not truly represent ergot, or perhaps the alcohol present, may have influenced his results.

Salant and Harris (3) noted that the temperature of chickens fell when fluid extract of ergot was given during acute alcohol intoxication, "thus indicating a reversible action, since in the normal subject the injection of ergot frequently caused a marked rise of temperature.[2]

Dale (2) in his detailed description of the action of ergotoxin, mentions in several of the protocols, that there was a rise of

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[1] A proprietary preparation of ergot, containing 20 per cent alcohol.

[2] In a personal communication, Dr. Salant has since told me that this rise of temperature was always obtained in normal chickens if the alcohol were driven off from the fluid extract, before injection.

temperature, but he evidently considered it of secondary importance as he nowhere in the text refers to this action. In the protocols of several of his cocks, high temperatures are noted, the highest being 44.5°C., and in those of several rabbits the same phenomenon is mentioned. In a footnote to the protocol of a rabbit which showed the rapid and fatal rise, which will be described later, he remarks: "High temperature at and after death, seemed to be a characteristic of fatal doses (in rabbits)." This is the only indication in the entire article to show that the hyperthermia had attracted his attention.

In a careful search of the literature on the physiologic action of ergot, these three are the only articles I have been able to find in which an action on the body temperature is mentioned, and in one of them the action obtained was not that which has been found characteristic of the active principle of ergot, and the other two mentioned the hyperthermia only incidentally in connection with other actions of the drug, in which alone the authors were interested.

While studying the action of ergotoxin on the pupil of the rabbit, I noticed that those animals which had received large doses showed strong shivering and erection of the hair, and on taking the temperature it was found to be very high. More detailed studies of the action of ergotoxin on the temperature of rabbits were therefore made and the behavior of several other species was also studied.

The preparation used in all my experiments was the ergotoxin phosphate of Burroughs, Wellcome and Company. Rabbits, cats, rats and mice were studied and a few observations were also made on pigeons. The injections were given intravenously in the cats and most of the rabbits, and subcutaneously in the other species. The animals were left free in a box or cage at the temperature of the laboratory excepting in certain special experiments on rabbits. In all the experiments excepting those on cats, control animals were kept with those injected. The temperature of the controls was almost constant in the rabbits and never varied more than 1°C. in the rats and 1.5°C. in the mice.

In all the species studied, ergotoxin was found to have a pro-

found influence on the body temperature.   It is remarkable how-ever, that in certain species it causes a very extreme rise of tem-perature, and in others an equally decided fall.

The rabbits showed with all doses a rise of temperature, vary-ing with the dose, being always very high with toxic amounts and frequently reaching a fatal hyperthermia.   Cats also showed a rise, although this was less extreme.   The rats and the mice showed an exactly opposite effect.   Both with toxic and with small doses, the temperature fell sharply, reaching low levels in both species.   The few experiments on pigeons, showed that in this species there is a fall of temperature, although not enough experiments were made to determine the average fall from any given dose.

As the various species showed such a marked difference in their response, the behavior of each will be treated separately.

Rabbits.   Large doses cause, in this species, several charac-teristic reactions.   If a toxic but not fatal dose of ergotoxin (2 mgm. per kilo), is injected in a vein, the first symptom seen is a dilatation of the pupil, which comes on within a minute and may in a short time be almost maximal.   This is followed in about ten minutes by marked restlessness, the animals running around in the box, or stamping noisily and keeping the limbs in constant motion.   The motions increase in violence if the rabbit is held.   This extreme restlessness lasts for half an hour or less and as a rule is much less pronounced, before the temperature has reached a very high level.   About an hour after the injection, the violent respiratory movements which characterize the period of extreme restlessness, give place to quieter but very rapid respirations, and about the same time or even much sooner, it is noticed that the hair is beginning to erect.   Whether this pilomotor response is dependent on the temperature is uncer-tain, as Dale has shown that ergotoxin stimulates the pilomotor muscle directly.   An hour after the injection the toxic symptoms are usually at their height.   The rabbit half stands, half lies, with the forelegs spread wide apart and the hindlegs not well drawn up under the body.   The head may be drawn back or may sink forward until the nose rests on the floor.   The tem-

perature is rising rapidly. There is an appearance of anxiety and fear when approached or handled. There is marked hyperesthesia, the subcutaneous muscles jerking at the least touch, although the animal left to himself is quieter than half an hour before. Walking is difficult on account of ataxia, although there is no weakness, the animal can kick strongly and the legs are stiff and show almost constant twitching and jerking. Attempts to walk result in violent, incoördinated contractions which occasionally are strong enough to throw a rabbit out of the box. The respiration is panting and so rapid that it is often difficult to count. The hair is erected and the pupils dilated widely. An hour later, that is two hours after the injection, the nervous symptoms are less marked although the temperature is now higher, reaching its maximum about this time. Three or four hours after the injection, the only deviations from normal are the high temperature, the rapid respiration and the dilated pupils. All of these are normal next day.

Ten experiments of this sort were made, in which the rabbits were given 2 mgm. per kilo of ergotoxin in the ear vein, and allowed to run free in a large box. In eight of these the temperature rose to 42°C. or 42.5°C. the average rise was 3.4°C. The maximum was reached in about two hours, and the fall began half an hour later, the original temperature being reached six to eight hours after the injection. The other two rabbits showed the fatal hyperthermia which has already been mentioned. The temperature began to rise immediately after the injection and rose rapidly, reaching 43.6°C. in one and one-half hours, when death occurred. The convulsive phenomena were no more marked in these animals than in the others although they were distinguished by greater hyperpnea when the fever was approaching its height. Among all my animals four showed this type of response. Both sexes and different ages are represented.

It is well known that when rabbits are tied down, their temperature tends to fall, and four experiments were made to determine whether tying the rabbits down before giving the injection would also counteract the tendency to fever after ergotoxin.

Although the individual temperature variation was greater in these animals than in those running free, the temperatures reached about the same level and similar tremors of the limbs were noted. One of these animals showed a rise to 44.2°C. in an hour and a half when death resulted.

Four other rabbits were given the same dose subcutaneously to make sure that the rise of temperature was not dependent on the method of injection. All of these showed extreme motor unrest and marked hyperthermia although the maximum temperature was reached several hours after the injection. One of them died at the end of four hours, the temperature having reached 43.4°C. The average rise was 2.5°C. This result shows that hyperthermia results in rabbits whatever the method of injection.

The great muscular activity which characterized these rabbits suggested that the fever might be due to the muscular contractions. Five experiments were made to determine whether curare would prevent the rise of temperature. In all of these animals the temperature rose distinctly although the rise was not as great as in normal rabbits, the highest temperature being 40.7°, a rise of 1.5°, the average rise being 0.6° and the minimum 0.2°. The constant occurrence of the rise however indicates that the fever is not solely dependent on muscular overactivity.

The ability of ergotoxin to cause fever independently of the convulsive phenomena, was even more strikingly shown by animals which were allowed to run free but which received a smaller dose, namely 1 mgm. per kilo. This gave rise to none of the nervous effects characterizing the larger dose except the dilated pupil and the hyperpnea. The temperature, however rose in all the animals in spite of the absence of motor excitability. Of course the effect on the temperature could not be expected to equal that of a dose twice as large, but the discrepancy was not greater than is usually seen in other connections. The temperature rose in a few animals above 42° and almost always above 40.5°. The average rise was 2° as compared to 3.4° with twice the dose.

These observations agree with those made by U. Mosso (4) on

the fever caused by cocain. He found that cocain caused in rabbits a rise of body temperature, associated with increased heat production in the muscles, but this increase was not dependent on contraction as it was not prevented by curare. Mosso also states that rabbits given curare and strychnin showed an increased heat production in the muscles although paralysis was complete.

In order to gain further information in regard to the relative importance of the muscular contractions and the direct action of ergotoxin on the heat regulating mechanism, the heat centers of several rabbits were removed by decapitation and the animals kept alive by artificial respiration. An electric hot pad kept the temperature near normal. The heat of the pad was not altered after the injection. This was not given until one-half to one hour after the operation, when the rabbit's temperature had been constant for some time. Two mgm. per kilo was then given intravenously and was followed by the tremors and jerking, characteristic of intact rabbits. In spite of this the temperature fell in one animal and rose only a fraction of a degree in the others. This result also points toward the conclusion that ergotoxin causes the hyperthermia by a direct action on the heat regulating center rather than indirectly by muscular action.

The muscular contractions induced by ergotoxin are readily suppressed by etherization. In rabbits immobilized by ether in contrast to curare, an intravenous injection of 2 mgm. per kilo caused absolutely no alteration in the temperature. It is probable that ether exerts a certain paralyzing action on the heat regulating center. Mosso's observation that choral, another narcotic, prevents the rise of temperature which follows the administration of cocain in the intact rabbit, may be mentioned as probably analogous.

Protocol 1. Intact rabbit, 2 mgm. per kilo in vein

Rabbit 1. White male; weight 1.6 kilo.
9.10 a.m. Pupils 4.5 mm. diameter. Temperature 39.5°. Pulse
192. Respiration 144.

9.15 a.m.   Injected in right ear vein ergotoxin, 0.4 per cent, 0.8 cc. (2 mgm. per kilo).   Rabbit left free in box.

9.17 a.m.   Pupils 6 mm.   Pulse 240.   Respiration 300.

9.20 a.m.   Pupils 7.5 mm.   Hair beginning to erect.   Body shaken by the respiration.   Had trouble keeping forefeet under the body as legs slid forward and were pulled back again.

9.25 a.m.   Temperature 39.3°.   Respiration 200, shallow and panting.   Skin feels very cold, ears cold but not pale. Hyperesthesia beginning.

9.40 a.m.   Temperature 39.8°.   Pupils 8 mm.

9.52 a.m.   Temperature 40.2°.

10.05 a.m.   Was very restless.   Forelegs still slid forward and were jerked back constantly.   Hair erect.   Pupils 8 mm. Temperature 40.6°.   Respiration 240.   Pulse about the same rate.

10.20 a.m.   Temperature 41.0°.

10.50 a.m.   Temperature 42.1°.   Skin and ears feel less cold.

11.00 a.m.   Very restless and excitable.   Moving jerkily and constantly.

11.15 a.m.   Temperature 42.5°.

11.35 a.m.   Lying with hind legs partly out behind.   Forelegs spread wide apart.   Head well up.   Entire body jerked by spontaneous twitching which did not alter its position. Marked hyperesthesia.   Respiration too rapid to count. Pupils 8 mm.   Temperature 41.6°.

12.00 m.   Less prostrated; could walk fairly well but ataxically. Temperature 41.4°.   Respiration still very rapid.

12.30 p.m.   Temperature 40.6°.

1.45 p.m.   Pupils 7 mm.   Temperature 39.5°.   Still had marked hyperpnea, respiration about 300 per minute.   Heart somewhat slower.

5.00 p.m.   Temperature 38.8°.   Respiration and movements almost normal.   Pupil still somewhat dilated.

Synopsis.   After 2 mgm. per kilo in vein, strong tremors and rise of 3°.

Protocol 2.   Intact rabbit 2 mgm. per kilo in vein

Rabbit 9.   Gray female; weight 1.5 kilo.

12.10 p.m.   Temperature 39.1°.   Injected in ear vein ergotoxin phosphate 0.4 per cent, 0.75 cc. (2 mgm. per kilo).

12.11 p.m.   Had strong shivering and front legs slid forward keeping her in constant motion in efforts to stand.

12.13 p.m.   Had convulsive seizures of entire body with kicking motions of the hind legs. These were more violent when the front legs were held still.

12.15 p.m.   Condition same. Skin and ears felt cold. Hair began to erect.

12.20 p.m.   Twitching the same. Temperature 40.2°.

1.10 p.m.   Condition had changed little. Sat with forelegs spread out wide so that body rested on the floor. Constant tremor or shivering. Respiration rapid with wheezing noise on expiration and inspiration. Temperature 42.2°.

1.40 p.m.   Condition worse. Lying on side. Twitching constantly. Temperature 43.6°. A few whiffs of ether were given to make introduction of the thermometer possible but on withdrawing it the respiration and then the heart stopped and the animal died. Postmortem. Only lesion of interest was slight edema of the lungs.

Synopsis. After 2 mgm. per kilo in vein, convulsive tremors and rise of 4.5° ending fatally.

Protocol 3.   Intact rabbit, 1 mgm. per kilo in vein

Rabbit 19.   Gray male; weight 1.7 kilo.

1.39 p.m.   Temperature 38.9°. Injected in ear vein ergotoxin 0.4 per cent, 0.42 cc. (1 mgm. per kilo).

2.09 p.m.   Had had no symptoms except moderate hyperpnea. Not restless nor hyperesthetic. Pupils 8.5 mm. Temperature 40.1°.

2.39 p.m.   Temperature 40.3°.

3.09 p.m.   Temperature 41.4°.

3.39 p.m.   Temperature 41.7°.

4.09 p.m.   Temperature 40.7°.

4.39 p.m.   Temperature 41.1°.

5.25 p.m.   Temperature 40.9°. The rabbit had no toxic symptoms excepting the hyperpnea and the dilatation of the pupil.

Synopsis. After 1 mgm. per kilo in vein, no tremors, but a rise of 2.9°C.

Protocol 4.   Rabbit under curare.   Ergotoxin 2 mgm. per kilo in vein

Rabbit 5.   Gray male; weight 1.4 kilo.   Etherized and prepared for artificial respiration.

9.51 a.m.   Operation complete.   Temperature 38.7°.   Artificial respiration begun.

10.08 a.m.   Had had 3.36 mgm. of curarin and now had no spontaneous respiration.   Pupil 5 mm.   Temperature 38.0°. Heart 144.   Strong peristaltic waves in cecum.

10.20 a.m.   On stopping artificial respiration, spontaneous began. Injected in vein curarin 0.6 mgm. per kilo.

10.35 a.m.   Complete paralysis.   Heart 144.   Pupil 5 mm.

11.00 a.m.   Temperature 39.1°.   On stopping air, spontaneous respiration began.   In vein curarin 1.5 mgm. per kilo.

11.12 a.m.   Fully relaxed.   Pupil 5.5 mm.   Injected in ear vein ergotoxin 0.4 per cent, 0.7 cc. (2 mgm. per kilo).

11.12 a.m.   Pupil 10 mm.   Temperature 39.2°.   Apex plainly seen, regular 240 per minute.   Cecum quiet.

11.20 a.m.   Temperature 39.2°.   Slight twitch of diaphragm began.

11.40 a.m.   No change in condition.   Temperature 39.5°.   Pupil 9 mm.

11.48 a.m.   Convulsive twitching began.   Injected curarin 0.8 mgm. per kilo.

11.53 a.m.   Quiet.   Temperature 39.6°.

12.15 p.m.   Began to twitch.   In vein curarin 1.44 mgm. per kilo.

12.30 p.m.   Fully relaxed.   Temperature 40.0°.

1.00 p.m.   Moving legs a little.   Temperature 40.3°.   Injected curarin 1.5 mgm. per kilo.

1.30 p.m.   Began to move.   Temperature 40.7°.   Curarin 1.1 mgm. per kilo.   From this time the temperature remained almost constant until 3.55 p.m. when the rabbit was allowed to die.

Synopsis. ,Twitching prevented by curare but after 2 mgm. ergotoxin per kilo in vein, a rise of 1.6°.

Cats.   In cats under the influence of ergotoxin, we noted the same peculiar mixture of narcosis and excitement which is described by Dale (2) in his protocols.   The pupils, in contrast to those of the rabbits were strongly contracted, but the temperature curve corresponded to that of the rabbit, showing a rise.

Intravenous injections of 2 mgm. per kilo, caused in each of three cats a rise of temperature varying from 1.5° to 1.7°. The following protocol shows the behavior of two of them. The third showed more tendency to narcosis.

Protocol 5.   Intact cat; 2 mgm. per kilo in vein

Cat 1.   Old male; weight 3.7 kilo.

9.36 a.m.   Injected in vein, ergotoxin 0.4 per cent, 1.85 cc. (2 mgm. per kilo).   Released at once.   Temperature 38.0°.

9.55 a.m.   Pupils 2 mm. across.   Temperature 38.0°.

10.00 a.m.   Not fully conscious, but on least touch, especially of head, showed strong striking movements of foreleg.   At times had spontaneous striking motions without being touched.

10.15 a.m.   Pupils 1 mm. across.   Temperature 38.4°.   Striking motions were weaker and were only well elicited by touching ears.   They were entirely purposeless and not designed to hit any particular things.

10.50 a.m.   Awake but lay still if not disturbed.   Was excited by any noise and opened mouth wide and growled but if anything was put in the mouth did not bite it.

11.20 a.m.   Lying quietly in cage.   Opened mouth and growled if approached but made no move to bite and was easily handled.   Pupils slits.   Temperature 39.0°.

12.20 p.m.   Condition same.   Pupils 2 mm.   Temperature 39.2°.

2.45 p.m.   Condition almost normal.   Temperature 39.5°.

4.30 p.m.   Recovered.   Temperature 39.2°.

Synopsis.   After 2 mgm. per kilo, narcosis and hyperesthesia, with a rise of temperature of 1.5°.

Rats.   In contrast to the rabbits, the rats showed neither motor excitement nor rise of temperature.   On the contrary they became quiet and seemed partly narcotized or better, stupid and dull, resisting handling less than the controls.   The temperature fell markedly.

With doses of 1 mgm. per kilo (about 0.3 mgm. per rat) the average fall was 2.9°.   As the average initial temperature was 38.5°, this represents a minimum temperature of 35.6°.   With a dose of 4 mgm. per kilo (above 1 mgm. per rat) the average

fall was more than 4°.   The initial temperature in these animals averaged only 37.5 and the minimum reached, ranged from 33.0° to 33.8°.

<center><i>Protocol 6.   Intact rat 1 mgm. per kilo subcutaneously</i></center>

<i>Rat 13.</i>   White male; weight 143 grams.

10.10 a.m.   Temperature 38.5°.   Injected subcutaneously ergotoxin 0.02 per cent, 0.72 cc. (1 mgm. per kilo).   Had no toxic symptoms except that he remained perfectly quiet if not disturbed and walked slowly and awkwardly.   The temperature was as follows.

10.10 a.m.   (Before injection) 38.5°.
10.40 a.m.   37.0°.
11.20 a.m.   35.8°.
12.30 p.m.   35.6°.
 1.30 p.m.   35.4°.
 2.30 p.m.   35.4°.
 3.30 p.m.   37.0°.

<i>Synopsis.</i>   After 1 mgm. per kilo subcutaneously, ataxia and fall of temperature of 3.1°.

<center><i>Protocol 7.   Intact rat 4 mgm. per kilo subcutaneously</i></center>

<i>Rat 10.</i>   White male; weight 95 mgm.

11.40 a.m.   Injected subcutaneously ergotoxin 0.4 per cent, 0.095 cc. (4 mgm. per kilo).   Soon became quiet and stupid and was easily handled.   Walked badly, dragging hind legs somewhat.   No other toxic symptoms.   The temperature range was as follows:

11.40 a.m.   (Before injection) 37.5°.
12.20 p.m.   35.3°.
 1.20 p.m.   33.8°.
 2.30 p.m.   35.2°.
 3.45 p.m.   34.6°.
 4.45 p.m.   35.5°.

<i>Synopsis.</i>   After 4 mgm. per kilo, subcutaneously, ataxia and fall of temperature of 3.7°.

<i>Mice.</i>   Seven mice were given ergotoxin subcutaneously in a dose of 0.002 mgm. per gram (corresponding to 2 mgm. per kilo).

None of these showed any toxic symptoms but in all there was a marked fall of temperature. The lowest point was reached within an hour and the fall averaged 3.3°. In those mice whose temperature reached the lowest levels, there was a loss of activity but this may perhaps be ascribed to the low temperature and not to any specific action of the ergotoxin.

Protocol 8.   Intact mouse 2 mgm. per kilo subcutaneously

Mouse 14.   White male, weight 24 grams.

2.16 p.m   Injected subcutaneously, ergotoxin 0.01 per cent 0.48 cc. (0.002 mgm. per gram). Showed little difference in behavior from controls in same cage, but moved about less.   The temperature was as follows.

2.10 p.m.   38.8°.
2.46 p.m.   33.5°.
3.46 p.m.   33.5°.
4.46 p.m.   35°.

Synopsis.   After 0.002 mgm. per gram subcutaneously became rather quiet and temperature fell 5.3°.

GENERAL SUMMARY OF EXPERIMENTS

Ergotoxin has, in all the species studied, a marked influence on the temperature. In rabbits and cats it causes a rise, in rats and mice on the contrary, a fall.

Rabbits receiving 4 mgm. per kilo intravenously show strong motor unrest and a very marked rise of temperature. In eight rabbits the average of the highest temperature reached was 42.5°C., representing a rise of 3.4°. Rabbits receiving the same dose subcutaneously, showed less motor disturbance but in eight experiments the temperature rose on the average to 42.0°C., representing a rise of 2.5°. Rabbits receiving 1 mgm. per kilo intravenously showed no motor excitement, but gave as the average of sixteen experiments a maximum temperature of 41.0°C., representing a rise of 2°. Rabbits tied down and receiving 2 mgm. per gram intravenously showed as the average of eight animals a maximum of 41.6°C., representing a rise of 2.7°. Rabbits given curare and receiving intravenously 2 mgm.

of ergotoxin per kilo showed a slight rise of temperature, the average maximum in five experiments being 40.1°C., representing a rise of 0.6°.   Rabbits under ether and rabbits decapitated and kept alive by artificial respiration showed no rise after 2 mgm. per kilo intravenously.

Cats receiving 2 mgm. per kilo intravenously showed a rise of temperature, the average maximum being 40.3°C., representing a rise of 1.6°.

Rats showed a marked fall of temperature after ergotoxin. With 4 mgm. per kilo subcutaneously eight rats showed an average minimum below 33.0°C., representing a fall of more than 4.0°.   With 1 mgm. per kilo the corresponding figures were 35.5°C. and a fall of 2.9°.

Mice also showed a marked fall of body temperature, the average minimum of seven animals receiving 0.002 mgm. per gram (2 mgm. per kilo) subcutaneously, being 34.4°C., representing a fall of 3.7°.

CONCLUSIONS

The facts reported seem to show that in many species, ergotoxin exerts a specific influence on the heat regulating center, disturbing the relation between heat production and heat dissipation.   In cats and in rabbits this induces a rise of body temperature; in rats, mice and pigeons it causes a fall.

The hyperthermia seen in rabbits, appears to be higher than that induced by any other drug.   Mosso (4, 5) who tested the action of many drugs on the body temperature, stated that cocain causes in rabbits, higher fever than any other drug, but he does not state in the only article to which I had access, (4) what were the temperatures he observed.   I therefore made some tests with cocain in order to compare it with ergotoxin.   In a series of rabbits given varying doses, including toxic and even fatal amounts, the highest temperature observed was only 41°C. and the average rise only half a degree.   As temperatures of 42.0°C. to 42.5°C. are constantly obtained with ergotoxin, this substance would seem to be peculiarly adapted to the experimental production of "aseptic fever."

The rise of temperature in rabbits appears to depend on an action on the center and not to be an indirect effect of the muscular tremors. This is shown by the constant rise in curarized rabbits, and by the failure to obtain a rise in decapitated rabbits, in spite of the occurrence of tremors.

The relative importance of heat production and dissipation, is not certain, as no calorimetric studies have been made. The coldness of the skin and ears in the rabbits suggest that there is reduced heat dissipation, and the tremors point as strongly to increased production. The extremely high temperatures reached also suggest that both factors are involved and combine their effects to produce the result. The absence of tremors in cats and the cold skin suggest that in this species, decrease of heat dissipation is the chief factor. The fall of temperature in rats and mice is perhaps partly due to the quietness and decrease of muscular activity, but this is entirely problematical and I prefer not to hazard an opinion.

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PHARMACOLOGICAL STUDIES OF THE IPECAC ALKALOIDS AND SOME SYNTHETIC DERIVATIVES OF CEPHAELINE

III. STUDIES ON PROTOZOÖCIDAL AND BACTERICIDAL ACTION

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A. AMEBACIDAL ACTION

Following the earlier work of Vedder (1) showing that emetine or ipecac is strongly amebacidal for water amebas, some experiments to compare the effects of emetine and cephaeline on these organisms were begun in 1913. Later the ethyl, propyl, butyl, amyl and allyl ethers of cephaeline were similarly tested.

The cultures were obtained by examining drops of a laboratory culture (obtained from Butler College) for amebas and when these were found the drop containing them was washed off the slide with 50 cc. of tap water into a small flask and 0.5 cc. of ordinary bouillon added. The flask was loosely stoppered with cotton and left at room temperature for several days or until a scum was noticed on the surface of the liquid. Active amebas were usually abundant in this scum at first but as a rule disappeared entirely from the culture in a week or ten days. As soon as amebas were found in the broth culture, loopfuls of this material were planted on the surface of agar plates and slant agar tubes. This agar was composed of agar, 20 grams, sodium chloride, 3 grams, beef extract, 3 grams, and water to make 1000 cc. It was 1 per cent alkaline to phenolphthalein.

The amebas multiplied quite readily at room temperature on this medium as long as it remained moist, but went into the

quiescent stage when it became dry.   It was found advisable to transfer the culture to fresh plates every four or five days in order to have actively motile organisms.

Numerous tests were made by placing a loopful of culture rich in amebas into a drop of emetine or cephaeline hydrochloride solution on a microscope slide and sealing a cover glass on with vaseline to prevent evaporation.   Controls in water were observed.

Protocol.   Culture from plate 14 containing very numerous actively motile amebas.   One loopful of this culture was placed in a drop of 1:50,000 solution of emetine hydrochloride and observed immediately, using a 4 mm. objective.

11.00 a.m.   Two active amebas in the field.
11.10 a.m.   Still active.
11.25 a.m.   Still active.
11.30 a.m.   Non-motile.   Round in outline.
11.45 a.m.   Non-motile.   Round in outline.
11.48 a.m.   One of them has disintegrated.
11.54 a.m.   The second has disintegrated.
12.00 m.   No other amebas found on searching the slide.

A loopful of the same culture placed in a 1:50,000 cephaeline hydrochloride solution showed disintegration of the amebas in the same time (fifty-four minutes).

In 1:10,000 solutions emetine and cephaeline hydrochloride were both actively amebacidal and destroyed all motile amebas in seven to twenty minutes.

In 1:50,000 solutions, emetine acted more rapidly than cephaeline in a series of tests and appeared to be more amebacidal. In greater dilutions (1:100,000) the results over short periods were inconstant and were not recorded.

In another series of experiments, bouillon cultures of amebas and solutions of emetine hydrochloride and cephaeline hydrochloride were mixed in definite proportions and allowed to stand in test tubes for one hour, after which agar plates were inoculated from them.   Forty-eight hours later control plates contained numerous active amebas while no amebas were found in the plates inoculated from the emetine or cephaeline hydrochlo-

ride solutions of 1:50,000, 1:100,000 and 1:200,000 strength. These same plates were examined four days later and all plates then contained active amebas.

This experiment was repeated using 1:200,000, 1:300,000 and 1:500,000 solutions of emetine and cephaeline hydrochloride and exposing the amebas to these solutions in test tubes for four hours. Plates inoculated from these solutions contained active amebas three days later.

This experiment was again repeated using 1:200,000 solutions and exposing the amebas to them for seven hours. Eight days later active amebas were present on all plates inoculated from these solutions.

To fifty hour cultures of amebas on agar slants were added 5 cc. of 1:50,000 solutions of emetine hydrochloride and cephaeline hydrochloride and these allowed to stay in contact for two hours. The solutions were then poured off and transplants made from the surface of the slant onto agar plates. All these plates contained amebas five days later.

In another series of experiments a drop of the drug solution of the desired dilution was placed on a slide and the ameba culture stirred into it, the exact time of the mixing being noted. The cover slip was then sealed in position and the preparation examined for amebas. If any were found they were carefully observed in order to note any changes they might undergo as a result of being acted upon by the drug. The amebas used in this series were grown in tap water to which beef extract had been added in the proportion of 1 part in 25,000. The drugs used were dissolved in distilled water. Proper controls were observed adding the cultures to distilled water. In this series emetine and the ethyl, propyl, and iso-butyl ethers of cephaeline were tested for amebacidal action. The following illustrates the method.

Dilution of Emetine:
1:1000. Six non-motile amebas were seen. No motility or change in shape occurred during twenty minutes.
1:5000. One ameba broke up in six minutes, another in nineteen minutes. Ten other non-motile amebas were seen.

1: 5000.   Two large, non-motile amebas were seen and remained mo-
     tionless for twenty-five minutes.
1: 8000.   One ameba broke up at end of twenty-two minutes, seventy-
     five other non-motile organisms were seen.
1: 10,000.   Seven amebas were seen, all sluggishly active.   One bal-
     looned out in eight minutes, another in seventeen, a third in
     twenty, a fourth in twenty-three, a fifth in twenty-five, and a
     sixth in forty.   The seventh was still sluggishly active after
     forty minutes.
1: 12,000.   Ten slides were examined and thirteen amebas seen.   These
     all ballooned out within two hours, most of them within thirty
     minutes.   Very few of them showed much activity.
1: 35,000.   Ten amebas were seen and all were actively motile at the
     end of fifteen minutes.

The results of this series may be thus stated, emetine hydro-
chloride and cephaeline propyl ether hydrobromide in dilutions
of 1: 1000, 1: 5000, 1: 8000, 1: 10,000 and 1: 12,000 were effective
in killing some of the amebas, while the changes induced in other
amebas were of such a nature that it was hard to decide whether
they were dead or merely in a resting or resistant stage.   Neither
of the above two drugs in 1: 35,000 caused the amebas to cease
their motility within fifteen minutes.   Cephaeline iso-butyl
ether hydrochloride and cephaeline ethyl ether hydrochloride in
1: 10,000 dilution killed some amebas and in others induced
changes that made the endpoint questionable while still other
amebas remained active for thirty minutes.

A second similar series was carried out in which the amebas
were obtained from agar plates and in which the drug solutions
were made up to 1: 1000 solutions in 0.85 per cent sodium chlo-
ride and were then made up to the desired dilution with tap water.
Emetine hydrochloride and cephaeline iso-amyl ether hydrobro-
mide were used in dilutions of 1: 1000, 1: 5000, 1: 10,000 and
1: 15,000.   Some of the amebas were broken up within a few
minutes, others remained round and quiescent, while some were
sluggishly active for considerable periods of time.   One slide
containing amebas in emetine hydrochloride, 1: 1,000, showed at
the end of an hour, 26 non-motile round amebas and 5 very

sluggishly active ones. In a 1:5000 solution of emetine hydro-chloride 22 slightly active amebas were found at the end of three hours. In a slide containing 1:10,000 emetine hydrochloride, 20 sluggishly active amebas were seen at the end of one hour. In a 1:15,000 solution of emetine hydrochloride 25 fairly active amebas were seen after one hundred and sixty-five minutes ex-posure. One slide of a 1:15,000 solution was examined seven-teen hours after preparation. Sixty-five non-motile round forms and the disintegrated remains of many amebas were seen. No motile amebas were present.

The following are observations made with cephaeline iso-amyl ether hydrobromide, given somewhat in detail but omitting many similar slides studied. See table on page 346.

In discussing these results it must be admitted that the effect of the ipecac alkaloids on water amebas varies greatly. In some cases the amebas are quickly destroyed as evidenced by their complete disintegration, in others they became round and re-main quiescent for long periods when it is impossible to say whether they are dead or not, and in still others they remain slug-gishly active for a long time. It is possible to have these three events take place in amebas from the same culture and even on the same slide. The process of disintegration does not always occur, and is really the only readily apparent indication of death. Disintegration is initiated by a motile organism first be-coming non-motile, assuming a round form, gradually swelling up or "ballooning out" and finally breaking up by rupturing and scattering particles of protoplasm in all directions. Other or-ganisms which do not rupture may become coarsely granular but as there are all degrees of this, it cannot be taken as an indi-cation of death.

Our experiments extended over a considerable period of time and it is possible that we dealt with different species of amebas. However, in general morphology they all appeared the same with the exception of one very small ameba which was undoubtedly a different species and which was more resistant to the alkaloids, and was disregarded in our experiments.

It seems evident from our plating experiments that some of

DILUTION	SLIDE	REMARKS
1:10,000	1	One round form was seen. The cytoplasm disappeared at the end of fifteen minutes leaving only the nuclear material and granular débris. Two active amebas were seen and these were still sluggishly active at the end of one hour
1:10,000	2	One round form was observed. At end of seven minutes the cell wall and cytoplasm suddenly disappeared leaving only the granular and nuclear material. The slide was examined again after seventy minutes, but only the disintegrated remains of amebas could be found
1:10,000	3	One ameba sluggishly changed its outline for fifteen minutes and then assumed a round form. During the next ten minutes some slight changes in outline occurred. The organism was quiescent during the following hour
1:10,000	4	No active amebas were present but quite a few round forms were seen. One of these broke up in fifteen minutes. Some granular material alone remaining. In searching the slide other disintegrated amebas were seen
1:15,000	5	One sluggishly changed its outline for five minutes and then assumed a round form. It remained quiet for three minutes and then disintegrated. Another one sluggishly changed its outline for fifty minutes, then assumed a round form, was quiet for seventeen minutes, and then broke up completely. No actively motile ones were seen at any time
1:15,000	6	Four amebas sluggishly changing their outlines were seen. One broke up in three minutes, another in six, another in nine and the fourth in fifty-seven minutes. Many disintegrated ones were found on searching the field at the end of the hour. No progressively motile ones were seen at any time
1:15,000	7	One changed its location slightly, and its outline sluggishly for thirty-one minutes and then assumed a round form. The granular material gradually became contracted leaving a clear zone all around it. No other changes were seen during the following sixty minutes
1:15,000	8	Many amebas were found. They changed their locations very little and their outlines sluggishly. At the end of five hours many were still present; some were round, some irregular in outline and some broken up; none were motile
1:15,000	9	One was active for two minutes, then assumed a round form and broke up after one minute. Another round form broke up four minutes after the slide was made
1:15,000	10	One round form broke up in two minutes and another in five. No active ones were seen
1:15,000	11	One round form broke up in three minutes. Six other very small amebas of a different species were sluggishly active after twenty-two minutes

the organisms are encysted and thus resist the action of emetine and cephaeline. For example cultures on agar were not destroyed in two hours by 1: 50,000 solutions nor in seven hours by 1: 200,000 solutions, although no active forms could be found immediately after this treatment and it required three or four days for them to develop on the agar plates. Our results are not strictly comparable with those of Vedder (1) who planted his cultures in a 5 per cent bouillon to which he added his ipecac solutions and allowed them to act for twenty-four, forty-eight and seventy-two hours. His dilutions however, were remarkably high, 1: 20,000 solution of F. E. Ipecac containing not more than 1: 1,000,000 of the ipecac alkaloids, emetine and cephaeline.

Wherry (2) tried the action of emetine on water amebas growing with symbiotic bacteria and concluded that emetine in 1: 20,000, 1: 100,000 and 1; 200,000 dilutions killed the amebas after twenty-three and one-half hours exposure but did not kill them in one hour.

One thing that has impressed us in all our work with these alkaloids is the fact that they act slowly. This is evident in their toxic, irritant, bactericidal and other actions. Minimal lethal doses cause death only after two to four days as a rule. Irritation when caused by placing emetine in the human eye does not manifest itself objectively or subjectively for four to eight hours. Bactericidal effects are more evident when the drug solution is allowed to act on the bacteria for a considerable time. So, too, the amebacidal effect is exerted slowly and while a very high dilution of these alkaloids will kill amebas when allowed to act over a long period, a much stronger solution fails to kill in a brief time. That this failure is not due entirely to encystment is shown by the maintenance of motility by these organisms while in contact with the alkaloids in solution.

B. ENDAMEBACIDAL EFFECTS

The slow and irregular amebacidal effect of the ipecac alkaloids in vitro was further confirmed by their action on the Endameba buccalis obtained from the pus of pyorrhea lesions. In these experiments a drop of normal salt solution, containing the

EMETINE HCl	SLIDE	REMARKS
1 : 20,000	1	One endameba sluggishly active for twenty-seven minutes when it assumed a round form and remained thus during the next thirty minutes
1 : 20,000	2	One active endameba assumed a round form in twenty minutes and remained thus for thirty minutes
1 : 20,000	3	One actively motile endameba was still active at end of one hour. At this time four other motile ones were found
1 : 20,000	4	One was actively motile at end of one hour
1 : 20,000	5	Six were actively motile at the end of sixty-five minutes
1 : 10,000	6	Three actively motile ones were observed for one hour. The slide was then examined and eleven other actively motile ones were found
1 : 10,000	7	Seven actively motile ones were seen eighty minutes after preparing the slide
1 : 5,000	8	One actively motile endameba observed for thirty minutes
1 : 5,000	9	One actively motile, assumed a round form in twenty minutes and remained thus for the ten minutes observed. Many similar round forms but no active ones were seen
1 : 5,000	10	One sluggishly active for twelve minutes when it became round and motionless
1 : 5,000	11	One actively motile endameba assumed an irregular form in fifteen minutes and remained thus unchanged for thirty minutes longer. It then became actively motile again and was still motile at the end of seventy-five minutes from the time the slide was prepared. Two other actively motile ones were found at the end of eighty minutes
1 : 1,000	12	Two endamebas were found. They were still actively motile after eighty-five minutes. One actively motile one came to rest in twenty minutes and during the following eleven minutes gradually assumed a round form. In looking over the field eighty-four minutes from the time of preparation, seven actively motile endamebas were seen
1 : 1,000	13	Twelve actively motile endamebas were seen at the end of ninety minutes. A few round forms were also seen
1 : 500	14	Two actively motile ones were seen. They came to rest after eight minutes and remained thus during an hour
1 : 500	15	One actively motile for one hour
1 : 100	16	One actively motile endameba was seen. Gradually became more sluggish and at the end of sixty-five minutes assumed a round form. This round form could still be seen after twelve hours
1 : 100	17	One endameba assumed round form in seven minutes and then remained unchanged during the following ten minutes of observation. A second endameba was actively changing its form and rather sluggishly its position at the end of one hour. A third endameba was changing its shape at the end of sixty-five minutes

EMETINE HCl	SLIDE	REMARKS
1 : 50	18	One sluggishly active endameba was seen and assumed a round form in five minutes and remained unchanged at end of fifteen minutes
1 : 50	19	No motile endamebas found
1 : 50	20	No motile endamebas found

CEPHAELINE PROPYL ETHER PHOSPHATE	SLIDE	REMARKS
1 : 20,000	1	One actively motile endameba found after seventy minutes. Many round forms seen
1 : 20,000	2	One sluggishly active for thirty minutes, then became round. Another sluggishly active for forty minutes then became round. Both remained so at end of one hour
1 : 20,000	3	One was sluggishly motile for thirty-three minutes and then remained quiet during an hour
1 : 12,000	4	Two sluggishly motile endamebas were killed in fifty-three minutes. Another showed sluggish motility for seventy-three minutes
1 : 12,000	5	Four were sluggishly motile during an hour
1 : 12,000	6	Five were sluggishly motile during forty minutes observation
1 : 10,000	7	Eleven actively motile endamebas were seen after one hour
1 : 10,000	8	Fourteen were still active at end of seventy-five minutes
1 : 5 000	9	One active at end of fifty minutes
1 : 5,000	10	One sluggishly active for one hour
1 : 5,000	11	One actively motile for three hours
1 : 5,000	12	One sluggishly motile for thirty minutes then became round
1 : 5,000	13	One actively motile for twenty-two minutes then became round
1 : 5,000	14	One, actively motile, was observed for an hour. The field was then searched and four other actively motile ones were found. One of these was still motile at the end of three hours
1 : 500	15	One active endameba came to rest and assumed a round form in twenty minutes and remained unchanged during another twenty minutes of observation
1 : 100	16	Eleven different slides were examined and in none were actively motile endamebas seen. In four of the slides some endamebas were found which were very slightly and slowly changing their outline but not their position. These also became quiescent within a few minutes
1 : 50	17	No active endamebas were found

CEPHAELINE ISO-AMYL ETHER HYDROBRO-MIDE	SLIDE	REMARKS
1:20,000	1	One actively motile endameba came to rest in five minutes and remained so for fifteen minutes. During the next twenty minutes it changed its outline but not its location. Forty minutes from the time the slide was prepared this endameba became actively motile again and continued so during the remainder of the observation period of seventy minutes. In looking over the field sixty to seventy minutes after the slide was prepared sixty actively motile organisms were counted
	2	Two endamebas were actively motile at the end of eighty-five minutes
1:1,000		Four slides were examined but no motile endamebas seen. Round forms were present

alkaloidal salt in the desired dilution, was placed on a microscope slide and into it was stirred a very small amount of pus taken from the bottom of a pyorrhea lesion. The cover slip was then sealed on with vaseline and the slide on a warm stage placed under the microscope and examined for endamebas. The effect of the drug was then observed and notes made as shown in the preceding tables.

These results are in harmony with those of Kolmer and Smith (3) who found that dilutions of emetine as strong as 1 in 800 did not kill the endameba of pyorrhea within two hours. These authors, however, state that when the action of emetine on endamebas is observed over longer periods (six hours) amebacidal effect is evident in dilutions as high as 1: 400,000. As our object was to compare the various cephaeline derivatives and as this method did not lend itself well to that purpose we did not attempt to confirm Kolmer and Smith's findings, but decided to test the relative protozoöcidal effects of these cephaeline derivatives on paramecia.

It may be well to add that the high endamebacidal action of emetine in weak dilution is well proven by clinical experience in administering this drug in endamebic dysentery and endamebic pyorrhea; and yet there are some instances in these diseases

where prolonged courses of emetine fail to eradicate the enda-
mebas from the lesions.   Most cases, however, respond promptly
to treatment and the refractory ones are probably due to en-
cystment of the organisms.

We have tested the endamebacidal action of emetine and
cephaeline adsorbed with Lloyd's Reagent (Alcresta Tablets of
Ipecac) given orally and in most cases have caused complete
disappearance of endamebas from pyorrhea lesions by giving an
equivalent of 60 grains of ipecac a day for six days.   This is in
accord with the findings of Bass and Johns (4).   We have also
tried with similar results, emetine hydrochloride, cephaeline
propyl ether phosphate, and cephaeline iso-amyl ether hy-
drochloride given hypodermatically in daily doses of $\frac{1}{2}$ grain.
Cephaeline iso-amyl ether hydrochloride was too irritating to use
in any number of cases, but its endamebacidal effect was very
evident in one case, who was given $\frac{1}{2}$ grain daily for four days.
On the first day 819 actively motile endamebas were counted in
two slides made from the pus on the point of one applicator.
The organisms rapidly decreased in number and on the fifth day
none could be found in the two slides examined.   On the seventh
day six slides were examined but careful search failed to reveal
any endamebas.

C. ACTION ON PARAMECIA

Vedder (1) noted the highly toxic action of emetine on para-
mecia and Sir Leonard Rogers (5) also used paramecia as test
organisms.   Rogers says,

In the first place, I found that the cephaeline salt had a less energetic
action on pathogenic amoebae in fresh dysenteric stools than the eme-
tine salt had.   It is however, difficult to obtain accurate comparisons
of the lethal action of the two alkaloids in this way because dysenteric
amoebae cannot be cultivated, and they tend to die out in dysenteric
stools within a few hours.   Moreover, when the amoebae cease moving
in the presence of the alkaloid, if it is not of sufficient strength to cause
changes in the structure of the organism visible under the microscope,
it is impossible to say if the organism has been killed or has entered a
quiescent stage, perhaps preparatory to becoming encysted.

.   .   .   .   I therefore tried the effects of the alkaloids on the common water paramecia, and found that high dilutions of the salts of both emetine and cephaeline caused these organisms to rapidly lose their motility, while many of them presented clear round spaces which soon became large bladder-like protrusions. When the solutions were added to water containing a large number of paramecia, they rapidly lost their movement and fell to the bottom of the tubes, and this change corresponded with the microscopical ones just mentioned and could be easily followed with a hand lens.

.   .   .   .   It does not necessarily follow that the action of these alkaloids on paramecia is exactly parallel with that on the amoeba dysenterica, yet the fact that I have found them to be in close agreement in their actions on these two forms of protozoa is suggestive of such a relationship and makes the results of my experiments worthy of record.

Paramecia are well adapted to this purpose as their death point is readily determined. Normally they are in constant motion, their cilia moving so rapidly as to be invisible when observed with a 6 mm. objective. As the ipecac alkaloid begins to affect the paramecia, they progress more slowly, their cilia move less rapidly and can readily be seen and the paramecia come to rest. Their cilia continue to move slowly and finally stop completely. If the organisms are observed a little longer, their bodies become further swollen and will as a rule abruptly rupture and disintegrate.

Paramecia are easily cultivated in tap water containing one gram of beef extract in 25,000 cc. In order to keep the organisms multiplying, this medium is changed or added to about once a week. Increased multiplication occurs on the addition of a few bread crumbs. The cultures used in our work had been propagated in the laboratory for two years.

In agreement with previous workers, we found that paramecia were sensitive to slight changes in their environment, so that they differed from day to day in their susceptibility to the action of emetine. In fact this variation may be remarkable as was exemplified by certain cultures tested on the same day. Culture 66 was killed by 1:20,000 solution of emetine phosphate within five minutes, whereas culture 67 was killed by the same

solution only after four hours exposure. Culture 67 had been made four days previously by adding 100 cc. of culture 59 to 900 cc. of tap water containing 1 : 25,000 beef extract and some bread crumbs. Culture 66 had been similarly made from culture 59 four days previously but no bread was added. Usually the variations are much less than this, though they are frequently pronounced. Therefore we used a solution of emetine phosphate as a standard for comparison in determining the relative protozoöcidal effect of each of the synthetic derivatives of cephaeline and also tested as many of then as possible on the same culture. All of these various alkaloids were tested as phosphates and their solutions made with tap water.

The method of procedure was to add by means of accurately graduated pipettes 0.5 cc. of the alkaloid solution to 0.5 cc. of the paramecia culture. These solutions were thoroughly mixed and then poured into two cells on specially constructed microscope slides, each cell having a capacity of 0.5 cc. Cover slips were then applied so that no air bubbles formed between the surface of the liquid and the cover slips. Each cell contained usually from 10 to 30 paramecia, the number depending on the richness of the culture used. The organisms could be observed easily with a 6 mm. objective and the exact time of their death noted. The time required to kill all the organisms in the cell furnished the figures for comparison; in other words, the most resistant paramecium in each cell was used as the test organism.

The tables on pages 354 and 355 show the tests made on two days, the 21st and 28th of August.

These tables illustrate the method used and show that individual paramecia from the same culture may vary considerably in their resistance to a given alkaloid, but that the comparative results of a number of tests are fairly concordant. The results of several series of such tests are summarized on pages 356 and 357.

Despite certain discrepancies appearing in the tabulation, it may be concluded that the higher homologues of this series are much more destructive to paramecia than either cephaeline or emetine. Indeed it would appear that cephaeline iso-amyl ether

Paramecia culture, 61, August 21

CEPHAELINE HYDRO-CHLORIDE	EMETINE PHOSPHATE	CEPHAELINE ETHYL ETHER PHOSPHATE	CEPHAELINE PROPYL ETHER PHOSPHATE	CEPHAELINE ISO-PROPYL ETHER PHOSPHATE	CEPHAELINE BUTYL ETHER PHOSPHATE	CEPHAELINE ISO-BUTYL ETHER PHOSPHATE	CEPHAELINE TERTIARY BUTYL ETHER PHOSPHATE	CEPHAELINE ISO-AMYL ETHER PHOSPHATE	CEPHAELINE ALLYL ETHER PHOSPHATE
1:20,000	1:20,000	1:20,000		1:40,000	1:40,000	1:40,000	1:40,000		1:20,000
2:13 / 2:28 – 15	1:54 / 2:08 – 9	2:25 / 2:35 – 10		2:42 / 2:54 – 12	2:54 / 2:57 – 3	2:17 / 2:21 – 4	3:02½ / 3:06 – 3½		3:22½ / 3:28 – 5½
2:13 / 2:32 – 19	1:54 / 2:06 – 12	3:37 / 3:45 – 8		2:42 / 3:00 – 18	2:54 / 2:59 – 5	2:17 / 2:21 – 4	3:15 / 3:19½ – 4½		3:22½ / 3:28 – 5½
4:04 / 4:26 – 22	3:26 / 3:42 – 16	4:08½ / 4:18 ÷ 9½	1:60,000 / 2:28 / :39 – 11	4:27½ / 4:41 – 13½	4:30½ / 4:34 – 3½				5:19½ / 5:32½ –13
4:04 / 4:26 – 22	3:26 / 3:39 – 13		4:23½ / 4:34 – 10½	4:27½ / 4:42 – 14½					5:19½ / 5:33 – 13½
			4:23½ / 4:33 – 9½		1:80,000 / 4:42 / 4:51 – 9	1:80,000 / 4:53 / 5:01 – 8	1:80,000 / 5:01 / 5:12 – 11	1:80,000 / 3:08½ / 3:13 – 4½	
					4:42 / 4:50 – 8	4:53 / 5:02 – 9	5:01 / 5:12 – 11	3:08½ / 3:11 – 2½	
								1:160,000 / 5:09 / 5:18 – 9	
								5:09 / 5:15 – 6	

Paramecia culture 69, August 28

EMETINE PHOSPHATE	CEPHAELINE PROPYL ETHER PHOSPHATE	CEPHAELINE ISO-PROPYL ETHER PHOSPHATE	CEPHAELINE BUTYL ETHER PHOSPHATE	CEPHAELINE ISO-BUTYL ETHER PHOSPHATE	CEPHAELINE TERTIARY BUTYL ETHER PHOSPHATE
1:40,000	1:40,000	1:40,000	1:40,000	1:40,000	1:40,000
9:43 / 10:27 – 44	9:33½ / 9:39 – 5½	9:39 / 9:51 – 12	2:44½ / 2:49 – 4½	2:52 / 2:58 – 6	3:01½ / 3:07½ – 6
9:43 / 10:12 – 29	9:33½ / 9:43½ – 10	9:39 / 9:53 – 14	2:44½ / 2:49 – 4½	2:52 / 2:58 – 6	3:01½ / 3:11 – 9½
9:55 / 10:30 – 35	10:37½ / 10:45½ – 8	10:41 / 10:57 – 16	1:60,000	1:60,000	1:60,000
10:49 / 11:23 – 34	10:37½ / 10:51½ – 14	10:41 / 10:54 – 13	3:27½ / 3:34½ – 7	3:31½ / 3:40½ – 9	3:38½ / 3:47¼ – 9
10:49 / 11:51 – 62	10:57 / 11:11 – 14	11:03 / 11:15 – 12	3:27½ / 3:35 – 7½	3:31½ / 3:43 – 11½	3:38¼ / 3:50 – 11½
1:57 / 2:33 – 36	10:57 / 11:03 – 6	11:03 / 11:23 – 20	3:46½ / 3:54 – 7½	3:59 / 4:06 – 7	4:04 / 4:18 – 14
	11:25½ / 11:34 – 8½	11:34 / 11:57 – 23	3:46½ / 3:54 – 7½	3:59 / 4:07 – 8	4:04 / 4:13 – 9
	1:55 / 2:06 – 11	11:34 / 11:54 – 20	4:18½ / 4:25 – 6½	4:21 / 4:34 – 13	4:34½ / 4:46½ – 12
	1:55 / 2:02 – 7	2:10 / 2:25 – 15	4:18½ / 4:26 – 7½	4:21 / 4:32½ – 11½	4:34½ / 4:48 – 13½
	2:07½ / 2:16 – 8½	2:10 / 2:34 – 24			
	:07½ / 2:15 – 7½				
	4:52½ / 5:03 – 10½				
	4:52½ / 5:01½ – 9				
Average 40 min.	10 min.	17 min.	4½ min. / 1:60,000 – 7¼ min.	6 min. / 1:60,000 – 10 min.	8 min. / 1:60,000 – 11½ min.

CULTURE	CEPHAELINE ALLYL ETHER PHOSPHATE	CEPHAELINE ISO-AMYL ETHER PHOSPHATE	CEPHAELINE TERTIARY BUTYL ETHER PHOSPHATE	CEPHAELINE ISO-BUTYL ETHER PHOSPHATE	CEPHAELINE N-BUTYL ETHER PHOSPHATE	CEPHAELINE ISO-PROPYL ETHER PHOSPHATE	CEPHAELINE PROPYL ETHER PHOSPHATE	CEPHAELINE ETHYL ETHER PHOSPHATE	EMETINE PHOSPHATE	CEPHAELINE HYDRO-CHLORIDE	DATE
59		1:100,000 3 min. 1:200,000 8 min. 1:300,000 11 min.							1:20,000 10½ min.		August 8
60							1:20,000 2⅔ min. 1:40,000 4 min. 1:80,000 11 min.	1:20,000 9½ min.	1:20,000 16⅔ min.		13
61			1:20,000 12 min.	1:20,000 7 min.	1:20,000 6½ min.	1:20,000 16⅔ min.	1:20,000 23 min.	1:20,000 41 min.	1:20,000 53 min.		15
61	1:20,000 17 min.	1:20,000 2 min. 1:40,000 5 min. 1:60,000 5⅔ min. 1:80,000 10 min. 1:160,000 16 min.	1:40,000 11 min. 1:30,000 8 min. 1:20,000 6 min.	1:40,000 8⅓ min.	1:20,000 10 min. 1:40,000 9 min.	1:20,000 18 min.	1:20,000 12 min.		1:20,000 30 min.		16
61									1:10,00 32 min.	1:10,000 37 min.	17
61		1:80,000 6 min.			1:40,000 4 min.	1:20,000 6 min. 1:40,000 14 min.	1:20,000 3 min. 1:40,000 6 min. 1:60,000 11 min.	1:20,000 10 min.	1:20,000 11 min. 1:10,000 8 min.	1:20,000 16 min. 1:10,000 12 min.	20

No.											Date
62	1:20,000 31 min.	1:20,000 2 min. 1:60,000 14 min. 1:80,000 14 min.	1:40,000 15 min.	1:40,000 7 min.	1:40,000 21 min.	1:40,000 34 min.	1:60,000 36 min.	1:20,000 33 min.	1:20,000 33 min.	1:10,000 34 min.	21
61	1:20,000 9½ min.	1:80,000 3½ min. 1:160,000 7½ min.	1:40,000 4 min. 1:80,000 11 min.	1:40,000 4 min. 1:80,000 8½ min.	1:40,000 4 min. 1:80,000 8½ min.	1:40,000 14½ min.	1:60,000 10 min.	1:20,000 9 min.	1:20,000 12¼ min.	1:20,000 19 min.	21
62					1:20,000 3 min. 1:40,000 4½ min.	1:20,000 5⅗ min.	1:20,000 4⅘ min.				23
62						1:20,000 7 min.	1:20,000 6 min.				24
69			1:40,000 8 min. 1:60,000 11½ min.	1:40,000 6 min. 1:60,000 10 min.	1:40,000 4½ min. 1:60,000 7¼ min.	1:40,000 17 min.	1:40,000 10 min.	1:40,000 40 min.			28
69		1:40,000 5¼ min. 1:60,000 7½ min. 1:80,000 11½ min.			1:40,000 6¼ min. 1:60,000 12 min. 1:80,000 11½ min.	1:40,000 4½ min. 1:60,000 12 min. 1:80,000 11½ min.	1:40,000 14 min. 1:60,000 16¼ min. 1:80,000 25½ min.		1:20,000 59 min. 1:40,000 62½ min.		29
75		1:40,000 4¾ min.			1:40,000 5⅔ min.				1:20,000 27 min.		Sept. 20
75		1:80,000 13 min.			1:80,000 18 min.				1:20,000 27 min.		21

phosphate is fifteen to twenty times as effective as emetine phosphate, whether this be measured by the time required for equal dilutions to kill or by the dilution of each required to kill in the same length of time. Thus on August 16 and 21, emetine phosphate was effective in thirty and thirty-three minutes, whereas cephaeline iso-amyl ether phosphate killed paramecia from the same cultures in two minutes. On August 8, a dilution of 1 : 300,000 of this last named salt killed paramecia of culture 59 in eleven minutes, whereas a dilution of 1 : 20,000 of emetine phosphate killed them in ten and one-half minutes. Cephaeline hydrochloride is certainly less active in this respect than emetine phosphate while both the ethyl and allyl derivatives are somewhat more toxic to paramecia than is emetine. The propyl, butyl and iso-amyl derivatives are certainly much more protozoöcidal to these organisms. In order to determine the relative efficiency of these three they were compared on August 29 using culture 69 and again on September 20 and 21 using culture 75. From these results, recorded in the above table, it is evident that these derivatives increase in activity in the order named. We have previously shown (6) that the substitution of the methyl group in emetine by radicals of the higher homologous alcohols decreases the toxicity when given subcutaneously to animals and it is of interest to note that this substitution markedly increases the toxicity for these protozoa.

D. BACTERICIDAL ACTION

The experiments of Vedder (1) showed that fluid extract of ipecac has an antiseptic action or inhibitory effect on the growth of B. typhosus, B. paratyphosus, B. dysenteriae, and Staphylococcus aureus. Wherry (2) testing the effect of emetine on amebas growing with a symbiotic bacillus, noted that the emetine in a dilution of 1 : 20,000 killed this bacillus in forty-eight hours. Price (7) found that emetine in 1 : 10,000 solution inhibited the growth of bacterial organisms taken from pyorrhea pockets and also had marked germicidal action upon them. He also stated that cephaeline had a similar but somewhat weaker bactericidal action. Kolmer and Smith (3), in a detailed study

of the bactericidal action of emetine, concluded that emetine possesses bactericidal properties, but that prolonged contact with bacteria is required before this action becomes apparent. They worked with B. typhosus, B. anthracis, B. subtilis, Staphylococcus aureus and Streptococcus salivarius.

Our experiments were carried out on cultures of Staphylococcus aureus with the object of comparing the germicidal action of emetine phosphate with cephaeline propyl ether and cephaeline iso-amyl ether phosphates. The method of procedure and results follow:

To each of three tubes containing 10 cc. of broth was added 2 cc., 1 cc., 0.5 cc. and 0.1 cc. respectively of a 5 per cent solution of each salt. Then 0.1 cc. of a twenty-four hour filtered staphylococcus aureus culture was added to each tube and mixed thoroughly, thus making dilutions of the alkaloidal salts of 1:121, 1:222, 1:424, and 1:2040. The tubes were incubated for twenty-four hours after which observations for growth were made. A transplant was made from each tube by streaking two loopfuls of the broth over the surface of a dextrose agar slant. This was repeated at twenty-four hour intervals for nine days. Control tubes were run by adding 0.1 cc. of the culture to each of three broth tubes. See pages 360 and 361.

Results obtained from the three control tubes indicate that after three days incubation the cultures lose vitality and eventually die. Emetine phosphate solutions of 1:121 are therefore not proven germicidal by these tests but apparently exert some inhibitory effect. Cephaeline propyl ether phosphate is germicidal in solutions of 1:222 and shows some inhibition in solutions of 1:424 for the first twenty-four hours but after that none. Cephaeline iso-amyl ether phosphate is germicidal in the weakest dilution used, namely 1:2040.

Additional tests on this last named substance were made in the same manner as before except that 20 cc. of broth and 1 per cent solution of the salt were used. Two sets of control tubes, one containing 10 cc. and the other 20 cc. of inoculated broth, were observed and incidently the loss by evaporation was noted from day to day.

Emetine phosphate

cc.	ONE DAY		TWO DAYS		THREE DAYS		FOUR DAYS		SIX DAYS		SEVEN DAYS		EIGHT DAYS		NINE DAYS	
	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar
2	3+	3+ Heavy	3+	3+ Heavy	3+	3+ 2 - I. C. 1 - 2 C	3+	3+ I. C.	3+	1+ 2- 1 - I. C.	3+	1+ 2- 1 - 1 C	3+	3-	3+	3-
1	3+	3+ Heavy	3+	3+ 2 Heavy 1 - I. C.	3+	3+ 2 - I. C. 1 - 3 C	3+	2+ 1 - 1 - I. C. 1 - 3 C	3+	3-	3+	3-	3+	3-	3+	3-
0.5	3+	3+ Heavy	3+	3+ Heavy	3+	3+ I. C.	3+	3+ I. C.	3+	1+ 2- 1 - 1 C	3+	3-	3+	3-	3+	3-
0.1	3+	3+ Heavy	3+	3+ Heavy	3+	3+ I. C.	3+	3+ I. C.	3+	3+ 1 - 1 C 1 - 7 C 1 - 9 C	3+	1+ 2- 1-12 C	3+	2+ 1- 1 - 1 C 1 - 1 C	3+	3-

Cephaeline propyl ether phosphate

cc.	ONE DAY		TWO DAYS		THREE DAYS		FOUR DAYS		SIX DAYS		SEVEN DAYS		EIGHT DAYS		NINE DAYS	
	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar
2	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-
1	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-
0.5	3-	2-1+ 1 - I. C.	3-	1-2+ 2 Heavy	1-2+	1-2+ 2 Heavy	1-2+	1-2+ 2 Heavy	1-2+	2-1+ 1- I. C.	1-2+	3-	1-2+	3-	1-2+	3-
0.1	3+	3+ Heavy	3+	3+ 2 Heavy 1 - I. C.	3+	3+ 1-3 C 2 - I. C.	3+	3+ 1-2 C 1-2 C 1-2 C	3+	3-	3+	3-	3+	3-	3+	3-

Cephaeline is -amyl ether phosphate

2	3-	3-	3-	3-	3-	3-	3-	3-·	3-	3-	3-	3-	3-	3-	3-
1	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-
0.5	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-
0.1	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-

Control tubes

3+	3+	3+ Heavy	3+ Heavy	3+	3+ I.C.	3+	3+ I.C.	3+ 2+1- I.C.	3+	3+ 2+1- 1-9 C 1-12 C	3+	3-	3+	3-

NOTE: No observations made on 5th day. "C" indicates colonies; "I. C." indicates isolated colonies too numerous to count. "Heavy" means heavy growth.

Cephaeline iso-amyl ether phosphate

	ONE DAY		TWO DAYS		THREE DAYS		FOUR DAYS		SIX DAYS		SEVEN DAYS		EIGHT DAYS		NINE DAYS	
	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar
2 cc. 1:1105	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-
1 cc. 1:2110	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-
0.5 cc. 1:4120	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-	3-
0.1 cc. 1:20,200	3-	2+1- Heavy	2+1-	2+1- Heavy	2+1-	2+1- Heavy	2+1-	2+1- I. C.	2+1-	3-	2+1-	3-	2+1-	3-	2+1-	3-

Control tubes

	ONE DAY		TWO DAYS		THREE DAYS		FOUR DAYS		SIX DAYS		SEVEN DAYS		EIGHT DAYS		NINE DAYS	
	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar
10 cc.	3+	3+ Heavy	3+	3+ Heavy	3+	3+ Heavy	3+	3+ I. C.	3+	3+ I. C.	3+	3+ 1-5 C 1-2 C 1-4 C	3+	3+ 1-6 C 1-3 C 1-10 C	3+	3-
20 cc.	3+	3+ Heavy	3+	3+ Heavy	3+	3+ Heavy	3+	3+ I. C.	3+	3+ 2- 1 C 1- 2 C	3+	2 -1+ 1 C	3+	2+1- 2 C each	3-	2-1+ 1 C

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Cephaeline iso-amyl ether phosphate is germicidal in a dilution of 1 : 4120 but not in 1 : 20,200. In the two sets of control tubes, there was no marked difference in the spontaneous death of the organisms. The amount of evaporation in these tubes was considerable, averaging 0.189 cc. per day from the 10 cc. tubes and 0.275 cc. from the 20 cc. tubes.

A third similar test using higher dilutions of cephaeline iso-amyl ether phosphate was run, with 10 cc. of broth and a 0.1 per cent solution of the salt. Ten dilutions ranging from 1 : 10,000 to 1 : 100,000 were tried. The 1 : 10,000 and 1 : 11,000 dilutions were inhibitory to the growth of the organism up to three days, but were not germicidal.

From these few tests it can only be said that both the propyl and iso-amyl derivatives of cephaeline possess far greater bactericidal action than does emetine. The experiments were too few and too inconclusive to afford an estimate of the degree of this action.

CONCLUSIONS

Emetine hydrochloride in solution of 1 : 1000 when acting on water amebas for one hour, or in solution of 1 : 5000 acting for three hours, destroyed many of these organisms but was not uniformly amebacidal.

Emetine hydrochloride in solution of 1 : 200,000 in contact with cultures of amebas for one hour, four hours, or seven hours destroyed many amebas, but transplants from these cultures to fresh agar plates showed a retarded or delayed growth of amebas, due probably to the development of encysted or resistant forms.

Emetine hydrochloride in solutions as strong as 1 : 100 are not rapidly destructive to the endameba buccalis, in some cases not killing them in one hour.

The propyl and iso-amyl ethers of cephaeline are stronger than emetine as amebacides but their action on water amebas or the Endameba buccalis cannot be used satisfactorily as a comparative measure of this action.

Methylating cephaeline to form emetine is known to increase

the endamebacidal·action as well as the protozoöcidal action toward paramecia and the substitution of the methyl group by ethyl, propyl, butyl, iso-amyl, or allyl further intensifies this action.

The propyl, butyl and iso-amyl ethers of cephaeline possess much stronger protozoöcidal properties than the methyl ether (emetine).   Cephaeline iso-amyl ether phosphate was the most effective alkaloid of this group in killing paramecia, being fifteen to twenty times as active as emetine phosphate.

Tested on Staphylococcus aureus in the manner described, cephaeline propyl ether phosphate is germicidal in solutions of 1 to 222, and cephaeline iso-amyl ether phosphate in solutions of 1 to 4120.   Both of these derivatives are much stronger than emetine in germicidal action.

REFERENCES

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CHANGES IN RHYTHMICITY, IRRITABILITY AND TONE IN THE PURGED INTESTINE

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As is well known, it is the custom to prepare patients for surgical operations by purging them. Most surgeons have the idea that this puts the bowel in the best possible condition for the ordeal; others say that their greatly improved results without preparation prove that much of the post-operative nausea, vomiting, gas-pains, and intestinal paralysis is due to the purge. Certainly, we know that many people feel miserable and depressed for a few days after purgation; they have a great deal of gas, and the bowels are slow to resume their normal activities.

This work was undertaken with the hope of throwing some light on this important clinical problem. It seemed reasonable to suppose that if purgatives have either tonic or depressant effects on the gut, these effects should be demonstrable in excised segments beating in Locke's solution. We were interested also to see if any change could be shown in the gradients of rhythmicity, irritability, or latent period. One of us has suggested that the downward progress of food through the tract depends largely upon a gradient of muscle forces, of irritability, and rhythmicity; i.e., the upper part of the bowel not only contracts more powerfully under stimulus, but it reacts more promptly and beats more rapidly than do the parts lower down (1). The intestinal contents naturally move from the more active, irritable regions above, towards the more sluggish, less irritable regions below. The regular uninterrupted progression of material through the tract would depend largely on the smoothness of these gradients. If the purgative

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should happen to leave some regions more fatigued or more irritable than others, the gradient might be upset (2). Even a general uniform exhaustion of the muscle would be a contraindication to the use of purgatives before operation. Theoretically, purgation might be due to an increase in the steepness of the gradients, brought about either by a rise in the duodenal region or a drop in the colonic region.

<div align="center">TECHNIC</div>

Rabbits were chosen for this work because segments from their intestines, when immersed in warm aerated Locke's solution, beat more regularly than do those from any other animal so far studied. This makes it fairly easy to point out variations from normal in any particular rabbit. Such normals have been established in over one hundred rabbits through previous work by one of us. Another reason for choosing the rabbit is that, in spite of the small size of the animal, its bowel is almost as long as that of man. The small intestine in the rabbit measures between 3 and 4 meters in length; in man it is about 5 meters. The colon of the rabbit is about 1 meter in length, about the same as it is in man. The distinguishing feature of the rabbit's intestine is its large cecum, which is always full of macerating vegetable matter. In spite of conditions which are ideal for fermentation, the intestine of the normal rabbit contains practically no gas; the walls are tonic and grasp their contents firmly. The distal colon normally contains small balls of cellulose, so dried out that they float in water.

In the following experiments we have used the purgatives that are ordinarily employed in the preparation of operative patients: castor oil, magnesium sulfate, calomel, cascara and jalap. It may be objected that the dosage used has been too large for the size of the animal, but we do not believe this is a serious objection, for the following reasons. As stated above, the intestinal lengths in the rabbit and man are comparable, and it may be that this factor is of greater importance in determining the dose of a purgative than is the body weight. It will also be seen that for the most part we have used doses

which, for the rabbit, are mildly laxative. Doses smaller than these had no effect. Our results would probably have been far more striking if we had purged all of the rabbits to the extent that a patient is purged. Almost all the rabbits used for this work were taken from one lot. With a few exceptions noted, they were free from parasites. From time to time, one was killed and its segments used as controls. The drug was given by stomach tube usually about noon; and the animal was killed the next morning about nine o'clock. They were not fed after the purge was given. Animals of similar weight were kept as controls. Their cages contained generally from 10 to 20 scybalae, as compared with 50 to 150 from the purged rabbits. The animal was killed by a blow on the head, and the abdomen opened immediately. Five segments of intestine were removed from the following locations: (1) duodenum; (2) upper jejunum; (3) upper ileum midway between the pylorus and the ileocecal valve; (4) lower ileum opposite the appendix; (5) colon where it parallels the first portion of the duodenum. These segments were kept in Locke's solution at from 5° to 10°C. and pieces 3 cm. long were cut as needed. These five segments from different regions were caused to contract rhythmically together in a beaker containing 400 cc. of aerated Locke's solution at 38°C.

<center>RESULTS</center>

It will be seen from the protocols that there were five *thoroughly purged* animals and one which, from the size of the dose and the appearance of the viscera, ought to have been purged. All of these animals were apathetic and looked sick. The bowels of these purged animals were injected, full of fluid and gas; sometimes atonic and flabby, often irritable here and there, and inclined to contract down into hard white cords. When the excised segments were put into the warm Locke's solution, their contractions were weak and irregular, and they soon became fatigued. They were less sensitive to some drugs applied locally; in one instance, the usual dose of adrenalin had to be increased one hundred times to produce any effect.

TABLE 1

DATE	WEIGHT	DRUG	DOSE	DEGREE OF PURGATION	VITALITY	APPEARANCE OF THE ABDOMINAL ORGANS	BEHAVIOR OF THE EXCISED STRIPS (D=duodenum: J=jejunum: M=middle: I=ileum: C=colon)
1917 June 7	gm. 1500	Castor oil	10 cc.	No sign of purgation	Apparently normal	Adhesions present from an intraperitoneal injection at some former time. The bowel was apparently normal except for the presence of unusually small and soft fecal masses in the colon	1st set—All beat well. 2nd set—D. soon failed. M. showed poor amplitude. 3rd set—All beat poorly. C. hardly at all. 4th set—D and C beat very poorly; others hardly at all
June 12	1800	Castor oil	15 cc.	No sign of purgation	Apparently normal	Cecum was full of fluid and gas. The fecal masses of the terminal colon were smaller and softer than normal	1st set—All beat well except D. 2nd set—All started fairly well but soon quit. I. quit last with high tone. 3rd set—All but J. beat quite well. 4th set—D. hardly beat at all. J. and I. gave irregular curves with poor amplitude. M. and C. beat well
June 14	1350	Castor oil	20 cc.	Heavily purged	Depressed	Small intestine nearly empty. Cecum filled with fluid and gas. Colon empty distally and very tonic	1st set—All began to beat promptly, but soon failed. The weakness appeared first in M. and J.
June 6	1200	Castor oil	30 cc.	Heavily purged. Tail much soiled. Cage much soiled	Much depressed	The parietal peritoneum was reddened and the mesenteric vessels much congested. This injection was greatest opposite the ileum. The stomach contained gas, and the wall showed fine capillary injection. The cecum was reddened and inflated; contained solid matter. The colon was empty distally except for a little semi-fluid material	1st set—all strips began poorly and soon weakened. C. did not beat at all. M. quit almost immediately. D. was the best. 2nd set—Resembled the first set but were weaker

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Date	No.	Drug	Dose	Purgation	Condition	Autopsy findings	Heart strips
June 6	1300	Castor oil	30 cc.	Heavily purged. Cage much soiled and tail much soiled	Somewhat depressed	Intestines full of gas and somewhat hyperemic. Colonic contents softer than normal	1st set—All began poorly and soon got worse. D. was very weak. J. was best. D., M. and I. were irregular 2nd set—All very weak. I. and D. were the only ones left beating regularly after a few minutes
June 9	1650	Magnesium sulphate	10 gm. in 30 cc. water	Large number of scybalae in cage	Apparently normal	Bowel practically normal except for the presence of some gas everywhere	1st set—All beat well and had good stamina 2nd set—All began well but D. and J. soon lost in amplitude and became irregular 3rd set—All beat regularly and with good amplitude 4th set—All beat well
June 12	1800	Magnesium sulphate	15 gm. in 30 cc. water	About 50 scybalae present in cage	Apparently normal	Small intestine inflated with gas, very motile and irritable. Colon full of gas and conglomerate masses of 4-6 small scybalae	1st set—D. began poorly and quit in a few minutes. J. and M. were fair; I. and C. were weak
June 28	1650	Magnesium sulphate	20 gm. in 60 cc. water	60 scybalae present in cage	Apparently normal	Fluid present in the peritoneal cavity. Small intestine contained some gas. Colonic content unformed. Cecum filled with fluid and gas	1st set—D. showed large amplitude, but was irregular. I. was irregular. The other strips beat well 2nd set—J. was irregular. I. became tonically contracted, then practically ceased beating. The other strips beat well
June 7	2400	Magnesium sulphate	30 gm. in 40 cc. water	No sign of purgation	Animal prostrated	Stomach hyperemic. Small intestine and cecum filled with fluid and gas. The small intestine showed a capillary injection, the intensity of which varied as the distance from the pylorus	1st set—All were somewhat irregular except C. J. was best; M. poor; I. had poor amplitude 2nd set—D. beat well, the others poorly, and irregularly

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TABLE 1—Continued

DATE	WEIGHT	DRUG	DOSE	DEGREE OF PURGATION	VITALITY	APPEARANCE OF THE ABDOMINAL ORGANS	BEHAVIOR OF THE EXCISED STRIPS (D=duodenum; J=jejunum; M=middle; I=ileum; C=colon)
1917	gm.						
June 8	1450	Fluid extract cascara	2 cc.	No sign of purgation	Apparently normal	Abdominal organs apparently normal	1st set—All beat well with good amplitude 2nd set—All had poor amplitude. J. was worst 3rd set—J., M. and C. beat quite well; D. and I. were very weak
June 13	1750	Fluid extract cascara	4 cc.	No sign of purgation	Apparently normal	Stomach contained some gas; lower colon contained considerable gas as well as small hard feces crowded together	1st set—I. began well but soon became weak and irregular. C. was quite normal. D., J. and M. quit in a few minutes
June 28	1200	Fluid extract cascara	6 cc.	No sign of purgation	Apparently normal	Mesenteric vessels congested. Stomach contained a little gas. Small intestine empty; cecum nearly so (no gas). The colon contained normal feces and some gas	1st set—All strips beat with good amplitude and tone 2nd set—D. and M. were irregular
July 3	2000	Fluid extract cascara	8 cc.	No purgation	Apparently normal	Hyperemia of the whole intestine; some gas in the colon	In 1st and 2nd sets all segments beat normally
July 3	1500	Fluid extract cascara	8 cc.	No purgation	Apparently normal	Considerable engorgement of mesenteric vessels; some gas in the stomach. Cecal contents fluid. Colonic content normal	In 1st and 2nd sets all segments beat normally

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June 16	1750	Calomel	0.1 gm.	Well purged (about 40 scybalae)	Apparently normal	Cecum contained fluid and much gas; its walls were slightly hyperemic. Colon was practically empty of solid material and ballooned with gas	1st set—All beat very well with large amplitude and slow rhythm; J. alone failed becoming weak and irregular. 2nd set—All beat quite regularly. D. was a little irregular at first and weak later
June 19	1500	Calomel	0.1 gm.	Well purged (about 60 scybalae)	Apparently normal	Mesenteric vessels considerably engorged. The cecal contents were fluid; there was also a small amount of gas	1st set—All beat very well. I. was a little weak later. 2nd set—D. and C. beat fairly well. The rest had poor amplitude and very irregular tone. 3rd set—All had good amplitude. M. and I. rapidly lost tone.
June 21	1500	Calomel	0.1 gm.	Well purged (about 60 scybalae)	Somewhat depressed	Stomach and cecum contained some gas. The cecal contents were fluid	1st set—All began with good amplitude. J., M. and I. were irregular and J. soon became weaker. 2nd set—Amplitude was poor in all, but J., M. and I. were irregular. 3rd set—All started out very well. J. and M. soon failed, becoming weak and irregular. 4th set—D. had poor amplitude but was regular. M. began well but soon lost in amplitude. J. and I. were weak and irregular almost from the start

TABLE 1—Concluded

| DATE	WEIGHT gm.	DRUG	DOSE	DEGREE OF PURGATION	VITALITY	APPEARANCE OF THE ABDOMINAL ORGANS	BEHAVIOR OF THE EXCISED STRIPS (D=duodenum; J=jejunum; M=middle; I=ileum; C=colon)
1917 June 15	1200	Compound tincture jalap	4 cc.	Well purged, floor of cage covered with scybalae	Apparently normal	The colon contained few fecal masses and was distended with gas	1st set—All began well but soon grew weak and somewhat irregular. 2nd set—All but C. began well but they soon lost tone and amplitude. This loss was most marked in M. and I. C. did not beat at all
June 20	1300	Compound tincture jalap	4 cc.	Heavily purged (150 scybalae)	Somewhat reduced	The mesenteric vessels were noticeably engorged. Contents of small intestine and colon were normal except for the presence of gas	1st set—All but M. began well but soon lost tone and amplitude and became irregular. 2nd set—All but I. began well but soon lost tone and amplitude. C. beat well. 3rd set—All showed poor amplitude; some were irregular and they soon failed in amplitude
June 23	1650	Compound tincture jalap	8 cc.	Fairly well purged (50 scybalae)	Apparently normal	Parietal peritoneum somewhat injected. Colon contained gas and the scybalae were closely packed	1st set—D. and C. contracted well and had good tone. J., M. and I. showed early variations in amplitude. 2nd set—Strips beat well
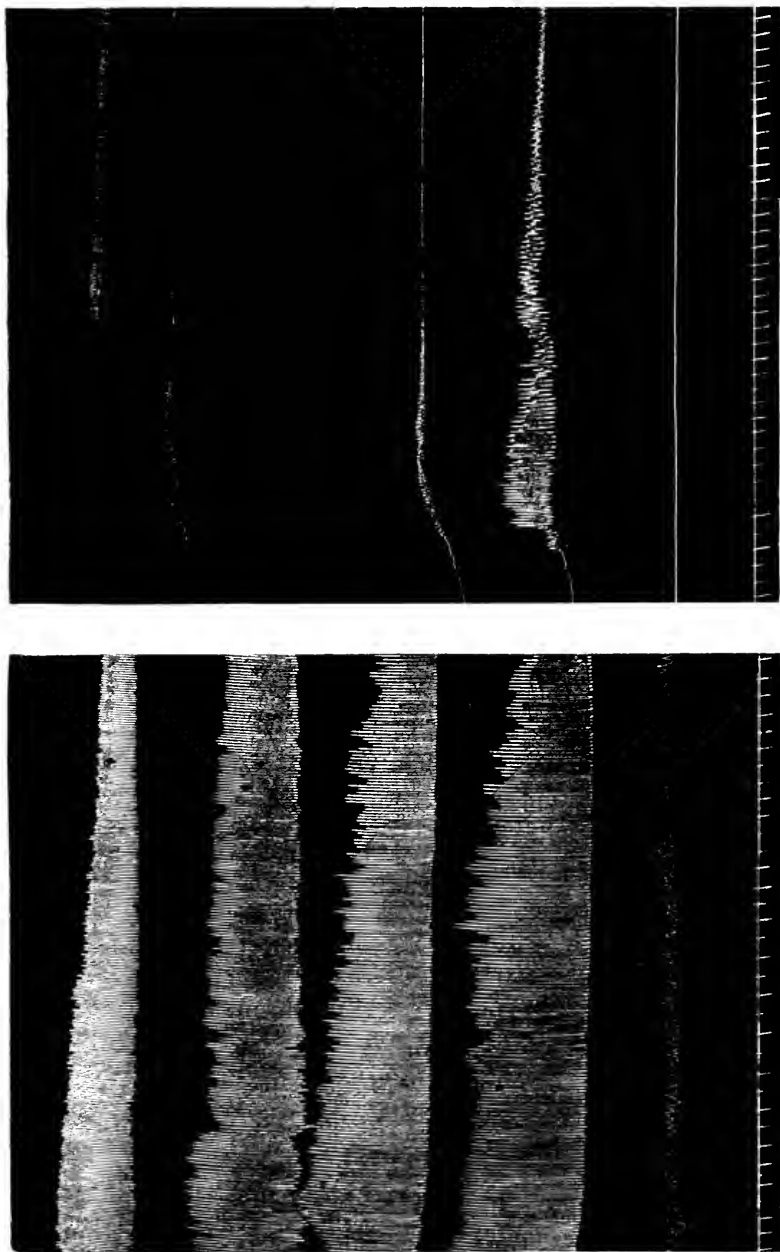
Fig. 1. Sample Tracings from Segments from a Normal Rabbit, on the Left, and a Rabbit Purged by Castor Oil on the Right

From above downwards the records are from the duodenum, jejunum, upper ileum, lower ileum and colon. The time record represents thirty seconds.

The importance of this observation will be evident to the man who knows how difficult it is to make the bowel respond to drugs after purgation.

Seven *moderately purged* animals gave no sign of prostration. All but one showed some intestinal gas or other abnormality. In four, the segments beat quite normally; in the other three, similar changes were observed as in the heavily purged animals.

Four rabbits showed *no sign of purgation until they were opened*, when the lower colon was found packed with softened feces. In three of these, the intestine was definitely abnormal and full of gas. In two, the segments beat poorly.

Three animals showed *no sign whatever of purgation.* One had some intestinal gas, and in two, the segments contracted poorly.

From these observations, magnesium sulfate would seem to be the most objectionable purgative for the surgeon. On account of its well known action in preventing the absorption of water by the bowel, the intestines in the animals purged by this drug were distended and full of fluid. It is interesting that although the lumen of the bowel in some of these animals still contained a large amount of magnesium sulfate, judging by the precipitate with barium chloride, not enough had gone through the thin mucous membrane to paralyze the muscular coat. Calomel and cascara did not seem to poison or fatigue the segments as did castor oil, magnesium sulfate, and jalap. With calomel, the segments beat well with a large amplitude and slow regular rhythm.

The gradient of rhythm in the excised segments was irregular only in the animals that received castor oil. The rate per minute was counted in several places on each record; and these data from all the sets from each animal were averaged for table 2. The final averages for the different drugs have been plotted in figure 2, together with a line representing the averages from forty normal animals. Although the data are not extensive enough for conclusions of any great value, the castor oil segments certainly beat faster and the calomel segments slower than the average. Probably these gradients would have been

found to be more upset if they had been studied in the intact intestine with the animals opened under salt solution.   Records obtained in that way from diarrhoeic animals showed very ir-

TABLE 2

Averages from the different sets from each animal

	RATE PER MINUTE					AVER-AGE
Castor oil						
Duodenum.........................	13.8	15.7	15.0	17.1	17.1	15.7
Jejunum...........................	12.5	13.7	12.3	15.1	15.8	13.8
Middle............................	13.2	13.7	13.5	14.9	12.0	13.4
Ileum.............................	11.4	12.7	11.0	12.5	12.5	12.0
Colon.............................		9.5	9.0	7.6	6.4	6.5
Magnesium sulfate						
Duodenum.........................	15.6	15.0	13.0	16.7		15.1
Jejunum...........................	14.6	13.5	10.3	14.7		13.3
Middle............................	13.7	9.0	9.1	13.4		11.3
Ileum.............................	11.6	7.5	10.1	12.6		10.4
Colon.............................	7.7	7.5	3.5			6.2
Cascara						
Duodenum.........................	14.5	15.5	17.0	14.5	14.0	15.1
Jejunum...........................	10.7	13.0	13.7	12.0	11.0	12.0
Middle............................	9.0	12.6	12.2	10.5	9.7	10.8
Ileum.............................	10.0	11.0	10.7	9.0	9.0	9.5
Colon.............................	9.0	12.0	6.5	6.0	5.0	7.7
Jalap						
Duodenum.........................	14.0	15.5	13.8			14.4
Jejunum...........................	12.0	12.5	11.8			12.1
Middle............................	11.5	11.0	10.0			10.8
Ileum......:.....................	10.0	10.0	10.1			10.0
Colon.............................	7.5	7.0	7.3			7.3
Calomel						
Duodenum.........................	14.0	12.3	14.7			13.7
Jejunum...........................	12.0	10.6	12.2			11.6
Middle............................	11.1	9.3	10.5			10.3
Ileum.............................	9.9	8.3	9.6			9.3
Colon.............................	6.5	7.0	6.2			6.6

regular gradients of rhythm (3).   When washed in Locke's solution and freed from disturbing influences, the excised segments tend to beat at their normal rates.

More striking deviations from normal were found when the latent periods of the segments were studied. Segments of intestine 2 cm. long were attached to a light heart lever and suspended in a moist chamber at 38°C. They were stimulated by a strong faradic tetanizing current. Normally, there is a cer-
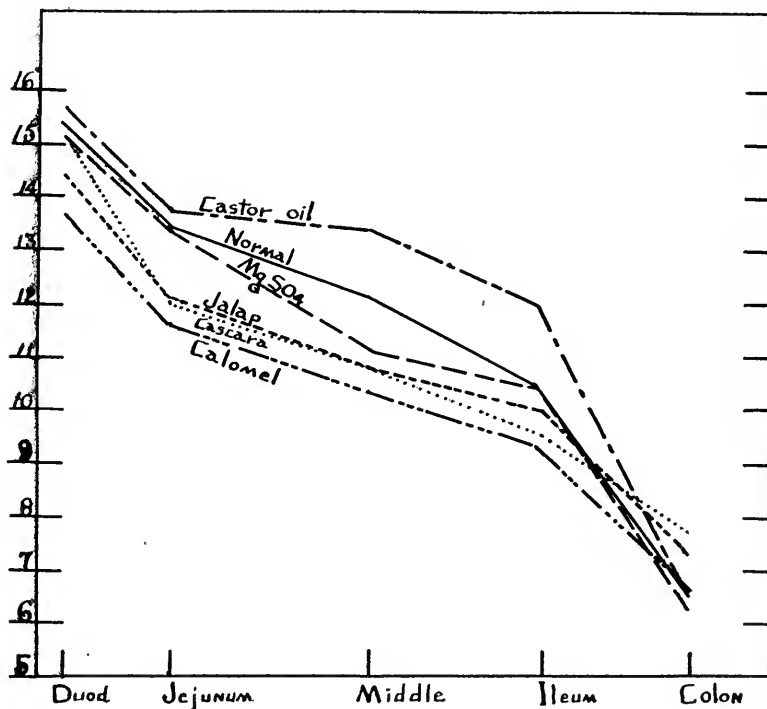
FIG. 2. AVERAGE GRADIENT OF RHYTHMICITY IN SEGMENTS EXCISED FROM PURGED AND NORMAL ANIMALS

The ordinates represent rates per minute, the abscissae distances from the pylorus.

tain gradation from the short latent periods in the duodenum and jejunum to the longer ones in the terminal ileum. In the purged animals, some segments were more irritable than normal, and had shorter latent periods; while others would hardly respond at all to the strongest current. We hope to publish the details of these experiments at another time. Colic and gas

pains might be due to the distension of such paralytic regions by gas forced into them and held there by more irritable and powerful loops above and below.   In the normal intestine, the gas if not immediately absorbed, would promptly move aborally because the oral end of the loop would be stronger, quicker and more irritable than the aboral end.

The injection of the intestinal wall, and the engorgement of the mesenteric vessels noted in many of the rabbits deserves mention.   It has been observed after purgation in man.   Such a disturbance in circulation might upset the delicate balance between the gases in the intestine and those in the blood.   As Schierbeck, Woodyatt and Graham (4) have shown, gas might even be exhaled from the blood into the bowel.

CONCLUSIONS

The well-purged rabbit is likely to be apathetic and to look sick.   Its bowel is full of gas and fluid, and the mesenteric circulation is disturbed.   Excised segments beat poorly and irregularly in Locke's solution, and they fatigue quickly.   They respond poorly to drugs.   Some parts of the bowel are abnormally irritable while others fail to respond at all to powerful stimuli.   This unevenness in the gradient of muscular forces must interfere with the steady progress of food through the gut; and probably favors the production of colic and gas pains. The conclusion drawn is that it is not wise to purge shortly before an operation in which the bowel must stand the insults of drying, handling, cutting and sewing.

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COMPARATIVE ACTIVITY OF LOCAL ANESTHETICS[1]
I. PARALYSIS OF MOTOR NERVE FIBERS

Direct Application to the Sciatic Nerve of the Frog

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CONTENTS

I. INTRODUCTION

The discovery of the numerous local anesthetics has naturally led to the wish to select "the best." Many attempts have been made to do this by laboratory methods. A review of the numerous papers show considerable discrepancies—some perhaps due to imperfect experimentation, many to failure to realize the limitations of a particular method, and therefore to the drawing of conclusions that the experiments do not justify. The clinical conclusions appear open to the same questions.

In view of the great importance of the subject, it seems worth while to reopen the more important and promising lines of investigation. I have therefore planned to compare those anesthetics that appear most useful in practice, or that have some .

other special interest; and to investigate these by methods and under such conditions as may be expected to throw a light on their specific advantages in the various fields in which local anesthetics are employed.

The clinical methods of the application of local anesthetics may be reduced for the present purposes, to three direct types:

1. Conduction anesthesia, i.e., application to nerve trunks, peripherally and in the spinal canal. This is reproduced experimentally by immersion of the nerve trunk in the solution. It involves the direct action of the anesthetic agent on the nerve fibers, with the minimum of complicating factors. The motor fibers have been used exclusively for these tests, because of technical convenience. It is possible, however, to extend the method to the sensory fibers, and thus to approach more closely to the clinical problem.

2. Terminal anesthesia, i.e., application to the smaller fibers in the distribution of the nerve. This is used clinically in the infiltration method. The same principles apply also to the hypodermic use. In these methods, the clinical efficiency depends not only on the action of the nerve fibers, but is complicated by the rate of absorption of the drug from the field of operation. Experimentally, this method is reproduced by the "wheal (quaddel) method" of intracutaneous injection.

3. Surface anesthesia, i.e., the application to superficial surfaces, especially mucous membranes (eye, nose, etc.). The efficiency here involves penetration through the membrane, as well as the action on the nerves and the removal by absorption into the tissues. Experimentally, the method is reproduced by the application to the cornea, or to the frog's foot.

The paralysis of the motor fibers by direct application to the sciatic nerve of frogs was selected for the first paper, because it is the simplest method and promises to give the most exact results. It would therefore be most likely to elucidate the principles. Its results, however, should not be transferred directly to the clinic, until they have been checked by other methods.

Critic of the motor-fiber method. The immersion of the nerve-trunk directly in the anesthetic solution reduces accidental dis-

turbances to the minimum. This method variously modified has therefore been used extensively, whenever exact comparisons were desired (Mommsen, 1881 (1); Laewen, 1906 (2); Gros, 1910, 1912 (3); Symes and Veley, 1911 (4); Hoffmann and Kochmann, 1912 (5); Zorn, 1913 (6); Closson, 1914 (7)). The sciatic nerve of the frog is usually employed.

Several possible fallacies have, however, been generally recognized: (1) in principle, it must not be overlooked that the method measures paralysis of the motor fibers. It is an open question as to how far it is justifiable to transfer these results to sensory fibers and nerves. It is known that sensory fibers are generally more easily paralyzed than motor fibers. It is not known whether the ratio of motor paralysis to sensory paralysis is the same for all anesthetics. Stovain, at least, is a departure from this rule (Santesson, quoted by Fromherz, 1914 (8)). The question can only by answered by experimentation.

(2) It must not be forgotten that experiments on exposed nerves cannot be transferred directly to injections or applications to mucous membranes, since the conditions of absorption are not reproduced.

(3) The criteria of paralysis are arbitrary and have been applied differently by different investigators. This makes it impossible to compare their absolute values, although the ratios should presumably be constant.

The absence of motor response to sciatic stimulation depends not only on the concentration of the solution and on the time of immersion; but also on the length of the immersed segment of nerve; on the size of the nerve; on the distance between the muscle and the stimulated point of the nerve; on the temperature; on the solvent; and on the strength of the current. These factors must be chosen arbitrarily.

(4) The anesthetics may be compared by either of the following methods: (a) by the time in which a given concentration produces paralysis; or (b), by the concentration that produces paralysis in a given time.

The first of these comparisons was used by Gros, Symes and Veley and Closson. It can be applied only with precautions.

It is true that within certain limits (fifteen to one hundred minutes), the paralysis occurs the more rapidly, the more concentrated the solution; but the relation is not a simple quantitative one, and may conceivably vary for different substances.

The second comparison was used by Laewen (one hour), and by Zorn (one-half hour). It also is not quite simple when two different substances are to be compared; for the ratio may be different for paralysis occurring in five minutes and paralysis occurring in two hours. In fact, however, my results indicate that the ratios appear to be practically the same for different periods.

Technic. The methods of every experimenter so far have differed so much that their results cannot be compared quantitatively. There was, consequently, no advantage in adhering to the arbitrary details of others. Nor was there any advantage in complicating the work by aiming at a theoretical degree of exactness of which the method was inherently incapable, and which would not have much practical importance. In this spirit, the following procedure was evolved, generally dictated by convenience:

The muscle-nerve preparations were made so as to include the lower end of the leg from the knee down, and the entire sciatic nerve from the knee to the spinal cord with a bit of bone attached.

The preparations were laid in $\frac{M}{8}$ (NaCl (0.73 per cent) made with tap-water. In making the tests, the entire nerve was immersed in the anesthetic solution, contained in a little trough cut into a block of paraffin. Each trough held about 1 cc. of solution. The excitability was tested with the platinum electrodes of a Harvard induction coil, activated by a current of about 4 volts, with the secondary at 12 cm. This stimulation is, of course, considerably above the threshold.

The stimuli were applied at the distal end of the nerve, that is, within 1 cm. of the spinal origin. Generally, when the block was completed at this point, response could be obtained by moving the electrodes half down the nerve; but this was disregarded.

The experiments were made at temperatures between 18 and 21°C. All the frogs (Rana pipiens) came in the same shipment.

The anesthetics were dissolved in $\frac{M}{8}$ NaCl (0.73 per cent); except that the concentrated solutions of antipyrin (4 per cent); KCl (1 per cent); NaHCO$_3$ (1 per cent); and Na$_2$HPO$_4$ ($\frac{M}{8}$) were made with water and diluted with saline.

The concentrations of the anesthetics were in geometric ratio ($\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 4, etc.).

II. RELATIVE EFFICIENCY OF THE ANESTHETICS ON MOTOR FIBERS

The mean of the observations is shown in table 1.

In table 2, the same data are arranged in another, perhaps more convenient manner. In the right half of the table, the "efficiency-ratio" has been calculated, cocain being taken as the unit. For instance, paralysis is produced in five to ten minutes by cocain, 1 per cent; novocain, 1 per cent; antipyrin, 4 per cent; and KCl, $\frac{1}{2}$ per cent. The novocain would therefore be as efficient as cocain (efficiency ratio = 1); antipyrin is $\frac{1}{4}$ as efficient (efficiency ratio = $\frac{1}{4}$); KCl is twice as efficient (efficiency ratio = 2), etc.

The efficiency ratios are shown for each period of time in which the paralyses were observed. The variations are so irregular, that they are probably accidental errors. The mean of all the observations is therefore given in the last column.

In brief, for the motor fibers of the frog's sciatic nerve immersed in the solutions, the relative paralytic efficiency (efficiency ratio) averages:

Cocain hydrochlorid
Novocain hydrochlorid
Tropacocain hydrochlorid
KCl 1

Alypin hydrochlorid.................................... $\frac{3}{4}$
Quinin urea hydrochlorid............................... $\frac{1}{5}$
Antipyrin.. $\frac{1}{8}$

In experimental work, for paralysis in thirty to forty-five minutes, the following were used as practically equivalent minimal effective concentrations:

TABLE 1

Time of paralysis of sciatic nerve

	PER CENT:								
	4	2	1	½	¼	⅛	1/16	1/32	1/64
	minutes	minutes	minutes	minutes	minutes	minutes	minutes	minutes	minutes
Cocain hydrochlorid			5 × 10*	× 5	20 × 30	30 × 45	45 × 60	90 ×	
Novocain hydrochlorid			5 × 10	10 × 15	15 × 20	15 × 25	65 × 90	90 ×	
Tropacocain hydrochlorid			10 × 15	15 × 20	15 × 20	20 × 30	35 × 45	120 ×	
Alypin hydrochlorid			15 × 20	15 × 20	30 × 45	30 × 45	90 × 120	120 ×	
Antipyrin	× 12	25 × 35	35 × 40						
Quinin urea hydrochlorid			45 × 65	45 × 65	45 × 65	60 ×			
KCl			× 4	5 × 10	30 × 45	15 × 65	90 ×	45 × 65	90 ×
NaCl $\frac{M}{8} = 1$			110 ×						
NaHCO₃			85 ×						
Na₂HPO₄ $\frac{M}{8} = 1$			85 ×						

* In this and all other tables, the numbers to the left of the × denotes the time (minutes) when there was no paralysis; and the number to the right of the × denotes the time (minutes) when paralysis was completed.

TABLE 2

Concentrations producing motor-paralysis in a given time

Drugs	TIME OF MOTOR PARALYSIS									RATIO OF EFFICIENCY (PARALYTIC CONCENTRATIONS) TO COCAIN = 1					
Minutes	0-5	5-10	10-15	15-20	20-30	30-45	45-65	65-90	>90	0-10	10-20	20-45	45-90	>90	Mean
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent						
Cocain hydrochlor.		1 and ½								1	1	1	1	1	1
Novocain hydrochlor.		1	½	¼	¼	⅛	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{32}$	½–1	1	1	1	1	1
Tropacocain hydrochlor.			1	½ and ¼	⅛	$\frac{1}{16}$			$\frac{1}{32}$	¼	½–1	2	1	1	1
Alypin hydrochlor.				1 and ½	⅛	¼ and ⅛			$\frac{1}{16}$ and $\frac{1}{32}$		½	½–⅛	¼–$\frac{1}{16}$	½–1	¾
Antipyrin		4		1 and ½	2	1			$\frac{1}{16}$ and $\frac{1}{32}$	¼		½–⅛	½–1	½–1	⅛
Quinin urea hydrochlor.						¼ and ⅛	1 to ¼		$\frac{1}{16}-\frac{1}{64}$			1	¼–$\frac{1}{16}$	¼	⅕
KCl $\frac{M}{8}$	1	½							⅛	1	1		½	½–2	1
NaCl $\frac{M}{8}$									$\frac{M}{8}$						∞
NaHCO₃									1						∞
Na₂HPO₄									$\frac{M}{8}$						∞

per cent

Cocain hydrochlorid ⎫
Novocain hydrochlorid ⎬ .... $\frac{1}{8}$
Tropacocain hydrochlorid ⎭
Quinin urea hydrochlorid.. 1
KCl... $\frac{1}{4}$

$\frac{M}{8}$ NaCl, NaHCO$_3$ and Na$_2$HPO$_4$ do not paralyze in the time of observation.

Results of other investigators. The data on the efficiency of the anesthetics on motor fibers may be compared by tabulating the concentrations that produce complete block in one-half hour. These are shown in table 3. The actual data vary widely, as was to be expected. The ratios in terms of cocain, however, agree quite well.

III. EFFECTS OF ALKALI

Gros, 1910–1912 (3), found that the addition of alkalies to the hydrochlorids of the anesthetic bases increases their efficiency very materially. He explains this by the liberation of the free bases, which penetrate the nerves much more readily than the water-soluble salts. He found further that the efficiency of the various salts of novocain is proportional to their hydrolysis; i.e., to the degree in which the free base is dissociated.

At first he added the amount of sodium bicarbonate calculated to bind the chlorid. He found, however, that an excess of alkali gave still greater efficiency, and therefore employed isotonic sodium bicarbonate or phosphate.

It is evident that these results cannot be transferred directly to the clinic. Granting that the free bases penetrate more readily than the salts, it must be remembered that in the clinical application, the anesthetics do not usually reach the normal fibers as pure salts, but that they are partly converted into carbonates by the alkalinity of the tissues. The importance of the alkalization of the solution would therefore presumably be smaller. This again is a question for investigation by other methods, and may be left for future consideration. For the present, it is important to check Gros' results.

TABLE 3

Comparison of investigators.

Minimal concentrations (per cent) of anesthetics that block the motor-fibers within one-half hour

	SYMES AND VELEY	GROS*	ZORN (WINTER)	ZORN (SUMMER)	CLOSSON	SOLLMANN	RATIO OF EFFICIENCY ON THE BASIS OF COCAIN = 1					
							Symes and Veley	Zorn (Winter)	Zorn (Summer)	Closson	Sollmann	
											Thirty minutes	Mean of all periods
Cocain hydrochlorid.....	$\frac{1}{15}$	$>\frac{1}{3}$	$\frac{1}{2}$	1	$\frac{1}{10}$	$\frac{1}{2}$	1	1	1	1	1	1
Novocain hydrochlorid..		1	$\frac{1}{2}$	$\frac{3}{4}$	$\frac{2}{10}$	$\frac{1}{8}$		1	$1\frac{1}{3}$	$\frac{1}{2}$	2	1
Tropococain hydro-chlorid..............			$\frac{1}{2}$		$\frac{1}{10}$	$\frac{1}{8}$		1		1	2	1
Alpin hydrochlorid......		$>\frac{1}{3}$	$\frac{3}{4}$		$\frac{1}{10}$	$\frac{1}{3}$		$\frac{2}{3}$		1	$\frac{1}{2}$	$\frac{3}{4}$
Beta Eucain hydro-chlorid......		$>\frac{1}{3}$	$3\frac{1}{4}$		$>\frac{1}{10}$			$\frac{1}{6}$		<1		
Stovain hydrochlorid....	$<\frac{1}{40}$	$>\frac{1}{4}$	$\frac{1}{2}$		$<\frac{1}{10}$		>3	1		<1		
Quinin urea hydro-chlorid...............					$\frac{1}{3}$	>1				$\frac{1}{3}$	$<\frac{1}{4}$	$\frac{1}{8}$
Antipyrin...............				$2\frac{1}{4}$		2			$\frac{1}{2}$		$\frac{1}{8}$	$\frac{1}{5}$
KCl....................				$\frac{1}{2}$		$\frac{1}{4}$			2		1	1
KNO₃...................				$\frac{1}{3}$					3			
K₂SO₄.................				$\frac{1}{4}$	$>\frac{1}{5}$				4	$<\frac{1}{2}$		

* Arch. Exp. Path. Pharm., 1910, 63: 80.

It was necessary, in the first place, to make certain that the alkalies, sodium bicarbonate and sodium phosphate do not have an anesthetic action. Table 1 shows that they are not in themselves anesthetic.

The following series of experiments were then made:

The solutions of the anesthetic hydrochlorids were mixed with 1 per cent $NaHCO_3$, or with $\frac{M}{8}$ Na_2HPO_4, so that the solutions contained:

PER CENT OF ANESTHETIC HYDROCHLORID	PER CENT OF $NaHCO_3$ OR RATIO OF $\frac{M}{8}$ Na_2HPO_4
$\frac{1}{8}$	$\frac{1}{2}$
$\frac{1}{16}$	$\frac{3}{4}$
$\frac{1}{32}$	$\frac{7}{8}$
$\frac{1}{64}$	$\frac{15}{16}$

In the case of cocain, another set of experiments was made by adding $NaHCO_3$ in the concentration of $\frac{1}{4}$ of the percentage of the cocain hydrochlorid, i.e., just about the amount required to bind the acid.

The detailed results are shown in table 4. The three series gave practically identical results, so that they may be grouped together, as in table 5.

TABLE 4

Effects of alkali on motor-nerve paralysis

(Time of paralysis in minutes)

	PER CENT OF ANESTHETIC			
	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$
Cocain with bicarbonate (just sufficient).	5×10	5×10	15×20	45×60
Cocain with bicarbonate (excess of bicarbonate).........................	5×10	10×15	20×30	20×30
Cocain with phosphate (excess of phosphate)...............................	10×15	$\times 5$	15×20	
Novocain with bicarbonate (excess ·of bicarbonate).........................	5×10	5×10	20×30	20×30
Novocain with phosphate (excess of phosphate)..........................	5×10	10×15	10×15	
Tropococain with bicarbonate (excess of bicarbonate).........................	$\times 5$	5×10	30×45	60×75
Tropococain with phosphate (excess of phosphate)..........................	15×20	30×45	45×65	
Alypin with bicarbonate (excess of bicarbonate)..........................	15×20	20×30	30×45	45×60
Alypin with phosphate (excess of phosphate)..........................	15×20	30×45	35×45	

The ratio of efficiency, as judged by the concentration required to produce paralysis in a given time, is increased as shown in the last column of table 5, namely: eight times for cocain and novocain; four times for tropacocain and six times for alypin. It is doubtful whether the differences are significant; it would probably be correct to say that all are potentiated four to eight times by the addition of alkali. This is in great contrast to potassium, which is not potentiated at all by alkali:

	$\frac{1}{4}$ *per cent*	$\frac{1}{8}$ *per cent*
Paralysis occurs in KCl:		
Without alkali...................	30×45 minutes	15×65 minutes
With alkali.....................	65×90 minutes	$90 \times$

TABLE 5

Effects of alkali. Comparison of mean results of hydrochlorids and base

(Time of paralysis in minutes)

	PER CENT OF ANESTHETIC:							EFFICIENCY RATIO: AS JUDGED BY THE CONCENTRATION REQUIRED TO PRODUCE PARALYSIS IN A GIVEN TIME, THE BASE IS X TIMES AS EFFICIENT AS THE HYDROCHLORIDE
	1	½	¼	⅛	⅟₁₆	⅟₃₂	⅟₆₄	
Cocain hydrochlorid...............	5 × 10	× 5	20 × 30	30 × 45	45 × 60	× 90	30 × 45	8
Cocain base.....				5 × 10	5 × 10	20 × 30		
Novocain hydrochlorid...........	5 × 10	10 × 15	15 × 20	15 × 25	65 × 90	90 ×		8
Novocain base...................				5 × 10	5 × 10	15 × 20	20 × 30	
Tropacocain hydrochlorid.......	10 × 15	15 × 20	15 × 20	20 × 30	35 × 45	120 ×		2 to 8, mean 4
Tropacocain base...............				5 × 15	10 × 30	30 × 65	60 × 75	
Alypin hydrochlorid.............	15 × 20	15 × 20	30 × 45	30 × 45	90 × 120	120 ×		4 to 8, mean 6
Alypin base.....................				15 × 20	20 × 45	30 × 45	45 × 60	

Comparison with the results of Gros. It is not possible to make quantitative comparisons with Gros' results, since he left the nerve in the solutions of the hydrochlorid for one and one-half to fourteen hours—which creates conditions quite different from those in my experiments. Qualitatively, my results confirm his conclusions, that the anesthetic bases are markedly more effective on the excised nerve than are their hydrochlorids.

IV. EFFECT OF EPINEPHRIN

H. Braun, 1903 (9), found that epinephrin increases very greatly the efficiency and duration of anesthesia in wheals, and in the clinical use. He attributes this to the vasoconstriction, preventing the absorptive removal of the anesthetic.

TABLE 6

Effect of epinephrin

	TIME IN WHICH PARALYSIS OCCURS AFTER IMMERSION IN			
	Cocain hydrochlorid		Novocain hydrochlorid	
	Concentration		Concentration	
	$\frac{1}{16}$ per cent	$\frac{1}{32}$ per cent	$\frac{1}{16}$ per cent	$\frac{1}{32}$ per cent
	minutes	*minutes*	*minutes*	*minutes*
Without epinephrin.........	45 x 60	>90	65 x 90	>90
With epinephrin............	65 x 90	>90	65 x 90	>90

The solutions contained epinephrin 1:20,000 for $\frac{1}{16}$ per cent of the anesthetic; 1:15,000 for $\frac{1}{32}$ per cent of the anesthetic.

Esch, 1910 (10), working under Kochmann, reports a series of experiments on the motor functions of the isolated mammalian sciatic nerve. He believes that these establish that there is also a more direct potentiation of cocain, novocain and alypin by epinephrin; tropacocain was not potentiated.

The results, however, are not very striking and not in themselves convincing. However, further work seemed desirable, especially since cocain and epinephrin are synergistic in some other respects. The results are shown in table 6.

Evidently, the paralytic action of cocain and novocain is not increased directly by epinephrin.

V. MIXTURES OF COCAIN WITH NOVOCAIN OR QUININ-UREA HYDROCHLORID

The question of the possible synergism of the anesthetics would have both practical and theoretical importance. It has so far been studied mainly by other methods.

When two anesthetics are combined, the effects may be the simple sum of their separate effects (simple summation); or they may be greater (potentiation); or smaller (negative potentiation).

When investigating this question, it is simplest to mix the anesthetics in the proportion of their minimal effective doses. For instance, the minimal effective doses to produce paralysis in thirty to forty-five minutes is, for cocain or novocain hydrochlorids, $\frac{1}{8}$ per cent; for quinin-urea hydrochlorid, it is 1 per cent. If solutions of this strength are mixed with each other, in any proportion, they will paralyze in thirty to forty-five minues if there is simple summation. If paralysis occurs in a shorter time, there is potentiation.

The degree of potentiation may be expressed in two ways; either (a) as the ratio in which the minimal effective doses of the constituents must be increased to produce paralysis in the same time as the mixture, which we may call the "Velocity Ratio;" or (b) as the ratio to which the mixture must be diluted to paralyze in the standard time of thirty to forty-five minutes, which we may call the "Dilution Ratio."

For instance, assume that paralysis is produced in thirty to forty-five minutes by anesthetic X in the concentration of $\frac{1}{8}$ per cent; and by anesthetic Y in the concentration of $\frac{1}{2}$ per cent. These are their minimal effective doses. Let us assme a mixture Z of equal parts of these solutions. This would contain $\frac{1}{16}$ per cent of X and $\frac{1}{4}$ per cent of Y. If there is simple summation, it should paralyze in thirty to forty-five minutes.

Let us assume that it paralyzes in ten to fifteen minutes. Let us assume that the concentration of X or Y necessary to paralyze in ten to fifteen minutes is four times that which paralyzes in thirty to forty-

five minutes (i.e., $\frac{1}{2}$ per cent for X or 2 per cent for Y). The potentiation by the velocity ratio is therefore 4.

We may also dilute Z until it paralyzes in thirty to forty-five minutes. Let us assume that this takes 3 parts of normal saline to 1 part of Z. This would show that Z is four times as strong as X or Y. The potentiation by the dilution ratio would also be 4.

TABLE 7

Mixtures of cocain with novocain (hydrochlorid)

PER CENT OF COCAIN	PER CENT OF NOVOCAIN	TOTAL PER CENT	TIME OF PARALYSIS	PER CENT OF UNMIXED ANESTHETICS THAT WOULD PARALLEL IN SAME TIME	EFFICIENCY RATIO BY VELOCITY
$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{8}$	65×90	$\frac{1}{16}$	$\frac{1}{2}$
$\frac{1}{32}$	$\frac{1}{32}$	$\frac{1}{16}$	>90	$\frac{1}{32}$	$\frac{1}{2}$
$\frac{1}{64}$	$\frac{1}{64}$	$\frac{1}{32}$	>90		

TABLE 8

Mixtures of cocain with quinin urea (hydrochlorid)

PERCENTAGE OF		TOTAL MINIMAL EFFECTIVE CONCENTRATION,* (30-45 MIN.)	TIME OF PARALYSIS	MINIMAL EFFECTIVE CONCENTRATION OF SINGLE ANESTHETICS REQUIRED TO PARALYZE IN THE SAME TIME		EFFICIENCY RATIO BY VELOCITY
Cocain	Quinin urea			Cocain	Quinin urea	
			minutes			
$\frac{1}{16}$	$\frac{1}{2}$	1	45×65	$\frac{1}{2}$	$\frac{1}{4}-1$	$\frac{1}{4}-1$
$\frac{1}{32}$	$\frac{1}{4}$	$\frac{1}{2}$	65×90	$\frac{1}{2}-\frac{1}{4}$	$\frac{1}{8}-\frac{1}{4}$	$\frac{1}{4}-1$
$\frac{1}{64}$	$\frac{1}{8}$	$\frac{1}{4}$	>90	$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{2}-1$

* The minimal effective concentration of cocain is $\frac{1}{8}$ per cent; of quinin urea, 1 per cent. A solution containing $\frac{1}{32}$ per cent of cocain and $\frac{1}{4}$ per cent of quinin urea would contain $\frac{1}{4}$ minimal effective concentration of each, or a total of $\frac{1}{2}$ minimal effective concentration.

In practice, the velocity ratio and the dilution ratio are generally not identical, and the velocity ratio may also differ according to whether one figures it from X or Y. The conclusions, in other words, are only approximate, i.e., within the range specified for each case, but they are sufficient for most practical purposes.

The results are shown in tables 7 and 8.

Neither novocain nor quinin urea show any potentiation with cocain. Indeed, there is not even perfect summation, but this is probably within the experimental error.

VI. MIXTURES OF ANESTHETICS AND POTASSIUM

Hoffmann and Kochmann, 1912 (5), found that mixtures of potassium and novocain give a well marked potentiation. Their results with the sciatic nerve indicate a potentiation of $2\frac{2}{3}$ by the dilution ratio, between novocain and potassiun. Their later work was done with the wheal method, and will be considered in another paper.

With the object of checking and expanding these data, series of experiments were made with mixtures of the individual anesthetics and potassium chlorid, by using the two anesthetics in the ratio of their "minimal effective concentration" thirty to forty-five minutes, i.e., the concentrations producing complete block in thirty to forty-five minutes, as stated below. The solutions were mixed so as to contain various proportions of the two ingredients, but with the minimal effective concentration unaltered; and these mixtures were then diluted to various degrees; the time of block being determined in each case. This was compared with the concentration of either agent alone that would give block in the same time.

For instance:

Cocain, $\frac{1}{8}$ per cent, paralyzes in thirty to forty-five minutes

KCl, $\frac{1}{4}$ per cent, paralyzes in thirty to forty-five minutes

A mixture of equal parts of these (therefore having the same minimal effective concentration = 1) paralyzes in five to ten minutes.

To produce paralysis in this time, would require:

Of cocain alone, $\frac{1}{2}$ to 1 per cent, i.e., four to eight times the total minimal effective concentration of the mixture, or a potentiation of four to eight times;

Of potassium chlorid alone, $\frac{1}{2}$ per cent, i.e., two to four times the total minimal effective concentration of the mixture, or a potentiation of two to four times. The potentiation acidity to velocity is therefore two to eight.

The mixture was diluted with normal saline so as to contain the following fractions of the original concentrations; and then produced paralysis in the following time:

$$\text{Dilution to } \tfrac{1}{2} \quad = 20 \times 25 \text{ minutes}$$
$$\text{Dilution to } \tfrac{1}{4} \quad = 45 \times 65 \text{ minutes}$$
$$\text{Dilution to } \tfrac{1}{8} \quad = 90 \times \qquad \text{minutes}$$
$$\text{Dilution to } \tfrac{1}{16} = 90 \times \qquad \text{minutes}$$

Consequently, the mixture had to be diluted two to four times to produce paralysis in thirty to forty-five minutes, i.e., the potentiation acidity to dilution was two to four.

Table 9 contains the detailed data, and table 10 the potentiation ratios deduced from them.

The results show quite marked potentiation, the efficiency of the mixtures being up to eight times as high as that of the unmixed components; thus confirming Hoffmann and Kochmann (5). The velocity ratios give higher figures than the dilution ratios. The most favorable results are observed if the minimal effective concentration ratio of potassium equals or exceeds that of the synergistic anesthetic.

VII. MIXTURES OF ANESTHETICS WITH BICARBONATE AND POTASSIUM

From a practical standpoint, it appeared interesting to determine the results of combining the synergism of potassium and alkalization. The results are shown in table 11.

The bicarbonate increases the efficiency of these cocain mixtures by two to four times; whilst it increases the efficiency of the cocain itself eight times. Since only a fourth of the mixture consists of cocain, this would correspond exactly to simple summation of the alkali and potassium, without any additional potentiation.

With the novocain mixtures, the results are not quite so favorable; but they probably fall within the experimental error.

It appears therefore that the beneficial effects of alkali on cocain are preserved but not increased in the cocain-potassium mixture; and this probably holds also for novocain.

TABLE 9

Mixtures of potassium and anesthetics on sciatic motor-fibers of the frog

RATIO OF MINIMAL EFFECTIVE CONCENTRATION OF SYNERGIST: KCl = TOTAL	PER CENT OF COCAIN, NOVOCAIN, TROPACOCAIN (HYDROCHLORID) (MINIMAL EFFECTIVE CONCENTRATION = $\frac{1}{4}$ PER CENT)	PER CENT OF KCl (MINIMAL EFFECTIVE CONCENTRATION = $\frac{1}{2}$ PER CENT)	TIME OF PARALYSIS IN MIXTURES				PER CENT OF QUININ UREA HYDROCHLORID (MINIMAL EFFECTIVE CONCENTRATION = 1 PER CENT)
			Cocain KCl	Novocain KCl	Tropacocain KCl	Quinin urea KCl	
15: 1 = 1	$\frac{15}{128}$	$\frac{1}{64}$	30 × 40				
7: 1 = 1	$\frac{7}{64}$	$\frac{1}{32}$	20 × 30	30 × 45	45 × 65		
3: 1 = 1	$\frac{3}{32}$	$\frac{1}{16}$	20 × 30	15 × 25	20 × 30	0 × 5	$\frac{3}{4}$
1: 1 = 1	$\frac{1}{16}$	$\frac{1}{8}$	5 × 10	10 × 15	10 × 15	0 × 5	$\frac{1}{2}$
1: 3 = 1	$\frac{1}{32}$	$\frac{3}{16}$	10 × 15 15 × 20	90 × 10 × 15	5 × 10	20 × 30	$\frac{1}{4}$
1: 7 = 1	$\frac{1}{64}$	$\frac{7}{32}$	× 5	90 ×	10 × 15		
1: 15 = 1	$\frac{1}{128}$	$\frac{15}{64}$	20 × 30				
1: 1 = $\frac{1}{2}$	$\frac{1}{32}$	$\frac{1}{16}$	20 × 25	60 × 95	65 × 90	65 × 90	$\frac{1}{4}$
1: 1 = $\frac{1}{4}$	$\frac{1}{64}$	$\frac{1}{32}$	45 × 65	90 ×	90 ×	90 ×	$\frac{1}{8}$
1: 1 = $\frac{1}{8}$	$\frac{1}{128}$	$\frac{1}{64}$	90 ×	90 ×	90 ×	90 ×	$\frac{1}{16}$
1: 1 = $\frac{1}{16}$	$\frac{1}{256}$	$\frac{1}{128}$	90 ×	45 × 65	90 ×	90 ×	$\frac{1}{32}$
1: 3 = $\frac{1}{2}$	$\frac{1}{64}$	$\frac{3}{32}$	30 × 45 30 × 45	15 × 20 90 ×	45 × 65		
1: 3 = $\frac{1}{4}$	$\frac{1}{128}$	$\frac{3}{64}$	45 × 65 45 × 65	65 × 90 90 ×	90 ×		
1: 3 = $\frac{1}{8}$	$\frac{1}{256}$	$\frac{3}{128}$	45 × 65 90 ×	90 × 90 ×	65 × 90		
1: 3 = $\frac{1}{16}$	$\frac{1}{512}$	$\frac{3}{256}$	45 × 65 90 ×				
1: 7 = $\frac{1}{2}$	$\frac{1}{128}$	$\frac{7}{64}$	× 5	90 ×	65 × 90		
1: 7 = $\frac{1}{4}$	$\frac{1}{256}$	$\frac{7}{128}$	90 ×	90 ×	90 ×		
1: 7 = $\frac{1}{8}$	$\frac{1}{512}$	$\frac{7}{256}$	90 ×	45 × 60	65 × 90		
1: 7 = $\frac{1}{16}$	$\frac{1}{1024}$	$\frac{7}{512}$	90 ×				
1: 15 = $\frac{1}{2}$	$\frac{1}{256}$	$\frac{15}{128}$	0 × 5				
1: 15 = $\frac{1}{4}$	$\frac{1}{512}$	$\frac{15}{256}$	30 × 45				
1: 15 = $\frac{1}{8}$	$\frac{1}{1024}$	$\frac{15}{512}$	75 × 90				

TABLE 10

Potentiation ratios of potassium and anesthetics

RATIO OF		POTENTIATION WITH							
Anesthetic (of percentage stated under each anesthetic)	KCl ($\frac{1}{4}$ per cent)	Cocain hydrochlorid ($\frac{1}{8}$ per cent) by:		Novocain hydrochlorid ($\frac{1}{8}$ per cent) by:		Tropacocain hydrochlorid ($\frac{1}{8}$ per cent) by:		Quinin urea hydrochlorid (1 per cent) by:	
		Velocity	Dilution	Velocity	Dilution	Velocity	Dilution	Velocity	Dilution
15	1	1				$\frac{1}{2}$–1			
7	1	1–2		1		1		4–x	
3	1	1–2		1–2		2–8	0	4–x	0
1	1	2–8	2–4	4	0	2–8	0	4–x	
1	3	1–4	2	$\frac{1}{4}$–4	2	2–8	0		
1	7	4–8	2	$\frac{1}{4}$	0				
1	15	1–2	4						

TABLE 11

Mixtures of cocain and novocain with potassium and bicarbonate

The anesthetics are adjusted so as to make the ratio of the minimal effective concentration of cocain or novocain hydrochlorid ($\frac{1}{8}$ per cent) to that of potassium chlorid ($\frac{1}{4}$ per cent) as 1 : 3.

COCAIN HYDRO-CHLORID	POTASSIUM CHLORID	TOTAL MINIMAL EFFECTIVE CONCENTRATION	TIME OF PARALYSIS WITHOUT ALKALI	TIME OF PARALYSIS IN PRESENCE OF ALKALI	RATIO IN WHICH THE MIXTURES ARE POTENTIATED BY ALKALI ACCORDING TO:	
					Velocity	Dilution
per cent	*per cent*					
$\frac{1}{8}$	0	1	30–45	5 × 10		8
0	$\frac{1}{4}$	1	30–45	65 × 95		>1
$\frac{1}{32}$	$\frac{3}{16}$	1	10–20			
$\frac{1}{64}$	$\frac{3}{32}$	$\frac{1}{2}$	30–45	15 × 20	4	2
$\frac{1}{128}$	$\frac{3}{64}$	$\frac{1}{4}$	45 × 65	30 × 45	4	2
$\frac{1}{256}$	$\frac{3}{128}$	$\frac{1}{8}$	65 × 90	30 × 45	4	4
$\frac{1}{512}$	$\frac{3}{256}$	$\frac{1}{32}$	65 × 90	90 ×		
NOVOCAIN HYDRO-CHLORID						
$\frac{1}{8}$	0	1	15–25	5 × 10		8
$\frac{1}{32}$	$\frac{3}{16}$	1	30–45			
$\frac{1}{64}$	$\frac{3}{32}$	$\frac{1}{2}$	20 × 90	30 × 45		2
$\frac{1}{128}$	$\frac{3}{64}$	$\frac{1}{4}$	65 × 90	90 ×	1	
$\frac{1}{256}$	$\frac{3}{128}$	$\frac{1}{8}$	90 ×	90 ×		
$\frac{1}{512}$	$\frac{3}{256}$	$\frac{1}{32}$	90 ×	90 ×		

VIII. CONCLUSIONS

1. Comparisons of the efficiency of local anesthetics must be adapted to the special uses of these agents. A complete review of the more important drugs, from this standpoint seems desirable. The present paper deals with the motor nerve fibers of the frog, and should not be transferred directly to clinical conditions.

2. With direct application to the motor-nerve fibers of the frog, cocain, novocain and tropacocain hydrochlorids and potassium chlorid are about equally efficient. The efficiency of alypin hydrochlorid is about three-fourths, quinin-urea hydrochlorid one-fifth and antipyrin one-eighth of the efficiency of cocain hydrochlorid.

3. Basic cocain, novocain, tropacocain and alypin are four to eight times as effective as the hydrochlorids. The addition of bicarbonate to potassium does not have this effect.

4. The addition of epinephrin does not increase the efficiency of cocain or novocain on the excised nerve.

5. The effects of mixtures of cocain with novocain or quinin-urea hydrochlorid do not show any potentiation.

6. The addition of potassium to cocain, novocain, tropacocain and quinin-urea hydrochlorid gives marked potentiation; the efficiency being up to eight times greater than would correspond to simple summation of the effects of the ingredients.

7. The potentiation by potassium holds also for basic cocain. That is, the potentiation by potassium and by alkali are simply additive.

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EXPERIMENTS ON THE THERAPEUTICS OF AMOEBIC DYSENTERY

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I. INTRODUCTORY

Amoebic dysentery was first transmitted from man to animals by Lösch (1) in 1875, who produced the disease in a dog. Later workers, beginning with Hlava (2) in 1887 and Kartulis (3) in 1891, have mostly used young cats for artificial infection. Infection of these animals with active amoebae introduced per anum, has now so often been achieved, that the procedure has even been suggested as an aid to diagnosis; for while Entamoeba histolytica in the active stage readily infects the mucous membrane of the cat's large intestine, producing a typical dysentery, there is no sound evidence to show that the harmless Entamoeba coli has ever been established in the cat.

There are also records of the infection of kittens by feeding them with the cysts of Entamoeba histolytica. Critical inspection of these shows that many are surprisingly unconvincing; but our own eventual success with this method, after a series of failures, leaves us in no doubt of the accuracy of previous conclusions. Our own aim was to obtain a transmissible and constant entamoebic infection, on which to test the action of substances already having a therapeutic reputation in amoebic dysentery, and of any others which seemed, theoretically, worthy of trial. We first observed the action of such substances on the amoebae in vitro, and then tested therapeutically, on infected kittens, those which had a definite lethal action on the isolated amoebae; but the intractable and rapidly fatal infections which

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we produced in kittens frustrated these latter attempts.   All that we were able to achieve practically, was a preliminary sorting, on the basis of their action on the isolated amoebae, of the substances tried.   This information, taken in conjunction with observations on the toxicity of these substances for mammals, furnishes hints of therapeutic possibilities, but nothing further.   The value of such indications can only be gauged by direct trial on cases of amoebic infection in man.   Some of the substances which our experiments indicated as at once safe, in respect of toxicity, and promising, in respect of amoebicidal action in vitro, are already being subjected to such clinical trials.   It will be seen later that the trials already completed give us reason for doubting altogether the apparent significance of the experiments made in vitro as regards curative action.

II. METHODS OF INFECTION

For maintaining an infection with Entamoeba histolytica in kittens, we used the method of injection of active forms per anum employed by most previous workers.   Baetjer and Sellards (4) have recently claimed greater certainty for a method in which the material is injected into the caecum by a hypodermic needle passed through its wall.   We have not tried this method, since we find no grounds for the advantage claimed.

It is possible, that greater regularity of infection is obtained by introducing the inoculum into the caecal end of the large bowel.   But the short, straight course of the kitten's large intestine makes it easy to do this with a glass nozzle of suitable length attached to the injection-syringe, and passed through the anus.   The only conceivable advantage of Baetjer and Sellards' method is that the small wound made by the needle in the mucous membrane might serve as a starting point for the amoebic infection.   They state, however, that this is not the case, and that the infection in their kittens so injected was limited to the lower end of the large intestine.   On the other hand, in most of our kittens infected per anum, the caecal mucous membrane was extensively ulcerated, as well as that of other parts of the large intestine.   As for certainty of infection, it may be noted that of the 32 kittens in Baetjer and Sellards' series, 24 were infected, while 8 failed to take the

infection.   But of the 24 successes, 16 were obtained with the rectal tube, and only 8 by injection through the wall of the caecum; while of the 8 failures, 6 resulted from injection through the caecal wall, and only 2 from use of the rectal tube.   If, then, we compare the percentage of failures with the two methods, as shown by Baetjer and Sellards themselves, we obtain the following result:

Trans-mural injection—6 failures out of 14, or 43 per cent.

Rectal tube injection—2 failures out of 18, or 11 per cent.

It may be further noted that Baetjer and Sellards, having carried the infection through 6 passages by trans-mural injection, thereafter employed the rectal tube for the 5 further passages which they achieved. Looking at our own results, and eliminating the cases in which infection was attempted with amoebae previously treated in vitro, and in which a negative result therefore indicated effective treatment, we find that, out of 139 kittens injected by the rectal tube, only 12 failed to acquire a fatal infection.   This number includes 2 which became slightly infected, but underwent spontaneous cure; 3 in which the result was compromised by the use of amoebae taken from a kitten some hours dead, or by the injection of a very small quantity; and three others which were simultaneously injected with a rather scanty emulsion of the strain which was lost by this failure to pass on the infection.

There can be no doubt, then, that the results which we obtained with the rectal tube show a greater certainty of infection than those obtained by Baetjer and Sellards by operation.   When the simplicity and safety of the rectal injection are further contrasted with the trouble of a method involving laparotomy, and its danger—witness Baetjer and Sellards' record of 5 deaths from general peritonitis in 14 experiments—the straightforward procedure appears obviously preferable.

Baetjer and Sellards attribute their success in maintaining a strain through 11 passages to their rule of using for injection material obtained from a kitten at the earliest stage of its infection.   We do not doubt that infection can be transmitted with these early dejecta, if sufficiently fresh.   But the amoebae very soon die after the mucus has been passed, and we found it much more convenient to kill our kittens soon after the appearance of the terminal symptoms of collapse, and to take material for transmitting the infection directly from the large intestine.

The large intestine was removed, laid on a glass slab, and split open. Any faecal material present was removed; and all the slime, with the ulcerated mucous membrane, was then scraped off with a spatula, and transferred to a bowl containing 25 to 40 cc. of saline solution. We used a 0.9 per cent solution of sodium chloride in London tap-water—the ordinary "physiological saline" of the laboratory. Baetjer and Sellards state that the amoebae are killed rapidly by a solution of such strength, and survive better in plain water or 0.2 per cent saline; but we found that the amoebae remained active and infective for 3 hours or more in the 0.9 per cent saline, while they swelled up and disintegrated in a few minutes if it was diluted with an equal bulk of distilled water.

For injection we used an ordinary "Record" syringe, of 10 cc. capacity. To the metal nozzle of this the glass rectal-tube was tightly attached with a short piece of rubber tubing. This tube was straight, 7 cm. in length, and 3 mm. in external diameter. The free end was rounded in the flame, leaving a small opening. A uniform emulsion of amoebae was obtained by repeatedly drawing the suspension of gut-scrapings in saline into the syringe and ejecting it again. Such an emulsion, prepared from a cat near the fatal termination of its infection, was usually crowded with active amoebae. For infecting a kitten we used 5 to 8 cc. of such a saline emulsion, according to its richness and the size of the animal. The syringe being suitably filled, and the kitten being held by an assistant, the end of the tube was gently passed through the anus. When the resistance of the internal sphincter was overcome, the tube passed easily into the large bowel. The animal was then held head-downwards and the piston pushed slowly home, so as not to provoke an expulsive contraction of the bowel. The tube was then withdrawn, and the anus kept closed for a minute or so by gentle pressure with the finger and thumb. This pressure was then removed, but the animal was kept head-downwards for a few minutes longer, to prevent the expulsion of the injection, and let the injected fluid percolate into the caecal end of the colon. The kitten was then returned to its

cage. A few drops of the injection were sometimes voided, but this small loss never seemed to prevent infection.

A strain of Entamoeba histolytica from human faeces containing active amoebae was established in kittens by similar injections. For infection with material containing cysts only, a smooth emulsion of the faeces was made in saline; 10 cc. of this was drawn into the syringe and injected directly into a kitten's stomach through a small soft-rubber catheter passed down the oesophagus. The emulsion was always first examined microscopically, to ensure that it contained a good proportion of living and mature cysts.

An unsuccessful attempt was made to infect a kitten by similarly introducing into the stomach an emulsion of active amoebae, which promptly and regularly infected when given per rectum. We also failed to infect kittens by injecting amoebae into the small intestine. For this purpose the animal was anaesthetized with chloroform and ether, and injection was made through the wall of an intestinal loop, exposed by a small laparotomy, with full aseptic precautions. In every case a diarrhoea resulted, but no amoebae or ulceration could be detected when the animal was killed a few days later; so that the diarrhoea was probably due to the incidental introduction of bacteria. One attempt was made to infect other organs by injecting an emulsion of living amoebae into the femoral vein, which was exposed aseptically under ether, and the tiny incision sealed with collodion. The kitten died on the 5th day after injection, probably from septicaemia, but no trace of an amoebic abscess was found in any organ post-mortem.

We used kittens about 6 to 8 weeks old, weighing 500 or 600 grams. Younger animals were less suitable, having less resistance and being unable to keep themselves clean. We found, as other have done, that older cats were less easily infected, even when an emulsion of amoebae was used which showed a high virulence for young kittens. Each animal was kept separately in a roomy wire cage, with a board placed on the floor for it to lie on. Each cage was supported over a zinc tray, into which faeces and urine dropped through the wire floor. The tray was slightly tilted, so that the urine ran down to one end, leaving the

faeces and mucus uncontaminated. The animals were inspected
each morning, and specimens of faeces taken for examination.
The trays were then removed, thoroughly scrubbed, and re-
placed. The food consisted of finely minced horse-meat and
milk—a ration on which the faeces of the normal kitten remained
formed and firm. The infected animals usually ate heartily,
remained vigorous and playful, and kept themselves clean, until
near the end of the infection. A failure to clean the fur around
the anus was frequently the earliest sign of the approach of a
fatal issue.

III. THE COURSE OF THE INFECTIONS

A. The strain obtained by infection with active amoebae from man

Infection of kittens with active amoebae from man was at-
tempted on two occasions, and succeeded once. The unsuccess-
ful attempt was made with freshly passed intestinal mucus
from a typical case of acute amoebic dysentery.[1] The material,
which was obtained before treatment was begun, was full of
active amoebae, and was emulsified in warm saline and injected
per anum into 3 kittens with the minimum loss of time.

The successful infection with active amoebae was carried out
with material obtained from a patient in Guy's Hospital, who
was suffering from a recurrent dysentery of subacute type. He
entered the hospital complaining of a persistent diarrhoea, and
active forms of Entamoeba histolytica were discovered in his
stools by Dr. G. W. Goodhart, to whom, and to Dr. P. P. Laidlaw,
we are indebted for samples of the infected faeces. The latter,
when examined microscopically by one of us, contained a small
amount of blood and mucus with active Entamoeba histolytica,
but no cysts. The material, which was not kept warm, was
emulsified after several hours with cold physiological saline, and
injected in 5 cc. doses into 2 kittens, no. 9 (on April 14), and no.
10 (on April 17). On April 18 each received a second injection.
The faeces of both were examined on April 22, and no amoebae
were then found. On April 23 both had passed some blood and
mucus, but no further microscopic examination was made till

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[1] We are indebted to Dr. G. C. Low for the opportunity of obtaining this
specimen.

April 25, when both were passing abundant blood and mucus, full of active amoebae of the typical histolytica type. No. 9 was found dead on April 26, but no. 10 survived until May 2, when it showed signs of collapse and was killed. Post-mortem both showed an extensive ulceration of the mucous membrane of the whole large intestine, and enlargement and inflammation of the corresponding mesenteric glands. In the liver of no. 10 a small resistant mass was felt, which caused a slight bulging of the surface. It was cut out and fixed in sublimate and acetic acid; and sections of it showed that it was an incipient abscess, containing numerous typical amoebae.[2] It is curious that in no subsequent passage of this strain of amoebae (over 40 passages and 120 kittens) was any trace of a liver-abscess detected, though regularly looked for post-mortem. Dr. Laidlaw informs us that a kitten which he similarly infected from the same patient also developed a liver abscess. The occurrence of this metastatic infection in 2 out of 3 kittens directly infected with the same human faeces, and its uniform absence in the long series of sub-infections, seems worthy of record, though we are unable to suggest any explanation.

Meanwhile several other kittens had been infected with material from nos. 9 and 10; and from these, in due course, yet others, by the method already described. Including the original infection with human material, 43 separate passages were carried out with this strain, which was maintained from April 25, when the initial infection was established, until December 2, when it was voluntarily abandoned. At the end of this period of more than 7 months, it was infecting as regularly as ever; and neither the course of infection, the time necessary for its development, nor the structure of the amoebae had undergone any appreciable change. There was evidently no natural limit to the propagation of this strain. Darling '(6) has stated that Entamoeba histolytica passes through a life-cycle, in passage through cats, changing from the "histolytica" to the "tetragena" and finally to the "minuta" form, when, with commencing cyst-

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[2] Wenyon (5) (1912) had previously produced a liver abscess, by similar experimental methods, in a kitten. There are also published records of the spontaneous occurrence of amoebic liver abscesses in the monkey, dog and badger.

formation, its infectivity by rectal injection is lost. We observed nothing in any way corresponding to such a metamorphosis in our 43 passages. We agree, therefore, with Wenyon (5), Baetjer and Sellards (4), and others, that a strain of amoebae in the cat remains constant and can be propagated indefinitely.

The 43 passages through kittens accomplished with our strain A appear to constitute the longest of such series on record. Brumpt and Chatton had previously attained 21 passages (vide Brumpt (7), footnote p. 29), and Baetjer and Sellards (4) reached 11. Quite recently Swellengrebel and Schiess (8) record 16 passages.

Baetjer and Sellards, contrary to most other workers, state that their infected kittens passed cysts of Entamoeba histolytica in addition to the free forms. Although we have examined a large number of kittens in the course of our experiments, we have never found a single cyst; and the authors' figures and descriptions of the "cysts" which they found fail to convince us of the correctness of their interpretation. Amoebic dysentery in kittens is always, in the experience of ourselves and most other workers, an acute affection closely similar to the acute condition in man; both being characterized by the passage of active amoebae unaccompanied by encysted forms. We find no good evidence that a true carrier condition accompanied by the passage of cysts, so commonly met with in man, is ever acquired by the cat.

Swellengrebel and Schiess state that in the cat Entamoeba histolytica possesses a nucleus differing in structure from that seen in amoebae from human infections. This is quite contrary to our experience, for we have always found the healthy amoebae in our kittens indistinguishable from those found in the stools of human beings suffering from acute dysentery.

We detected no increase of virulence in the infection with repeated passage, as described by Baetjer and Sellards. We may take as one criterion of virulence the number of days elapsing between the injection and the appearance of faeces containing blood, mucus and amoebae. This latent period was, on the whole, much shorter with this strain than with those studied by previous investigators. For the initial infection from human

material it is uncertain, as each kitten received two injections. Since the infection apparently started simultaneously in the two kittens, we may reasonably date it from the second injections, which were given simultaneously. If we take the earliest date at which blood and mucus were reported, the latent period was 5 days, though the first microscopic examination at which amoebae were found was made on the 7th day after the second injections. In the next passage, carried out with material from these two kittens, we note a shortening of the incubation period, 5 kittens so infected beginning to pass amoebae in the faeces, 3, 2, 2, 4 days and 1 day respectively after the injections.

But the apparent shortening loses its significance when we consider the manner of infection. The original two kittens received an emulsion of human faeces some hours old, containing relatively few amoebae. Four kittens were infected from this first pair with emulsions of freshly passed mucus, full of amoebae; while a fifth, with an incubation period of 1 day only, was given an emulsion of material scraped from the intestine immediately after death, and crowded with active amoebae. Thenceforward this last method was used whenever possible, and throughout the later passages the incubation period was usually 1 or 2 days. When a kitten on which we depended for passage died during the night, so that many of its amoebae were dead or degenerate, or when an emulsion proved unexpectedly poor in amoebae, a group of longer incubation periods sometimes followed. There was, however, no indication at all of a steady loss or gain of virulence. In 106 kittens infected with the first strain the apparent incubation-period varied from 1 to 6 days, and the different periods occurred with the following frequencies:

Length of incubation	Number of cases
1 day (or less)	37
2 days	39
3 days	19
4 days	5
5 days	5
6 days	1
	—
	106

Average incubation period: just over 2 days.

About the 8th and 9th passages a group of longer incubation periods occurred with two cases of spontaneous cure; so that the strain appeared to be losing virulence.   This was recovered com-pletely, however, at the next passage.

As another criterion of the virulence of the infection we may take the duration of the disease, from the appearance of the first evidence of infection until death.   As mentioned above, it was our practice to kill our kittens so as to obtain an abundance of vigorous amoebae.   Since, however, we never killed them until their condition indicated that death would probably occur within a few hours, there is no need to exclude these cases from a calculation in which the smallest time-unit considered is the day.

Time between onset and death	Number of cases
1 day (or less)	4
2 days	20
3 days	21
4 days	32
5 days	16
6 days	6
7 days	1
8 days	4
10 days	2
	106

Average duration: just under 4 days.

The short and long duration-periods occurred with great irregularity, so that the duration of the disease, like its incubation period, was probably determined by the resistance of the animal and the number of healthy amoebae in the infective emulsion, and not by change in the virulence of the strain.   The average incubation-period and duration in the 2nd to 5th passages were 1.8 and 4 days respectively, in the 40th to 43rd passages 1.5 and 4.2 days.

The first indication of infection with this strain was, in almost all cases, the passage of a small quantity of mucus and blood containing amoebae.   In a very few cases such small amounts of blood-stained mucus were passed each day, together with normal faeces, and the infection then underwent spontaneous cure.   In

all but these the quantity of mucus increased and the admixture of faecal matter diminished; the blood also, in many cases, almost disappeared on the last day or two of the infection, when there was an almost continuous dribbling from the anus of a thin, white or pinkish slime, crowded with active amoebae. The onset of fatal symptoms was often almost sudden. The kitten, which had been previously climbing actively about its cage, would be found somnolent and weak. The body temperature would then be found low, and the heart-beat slow and rather feeble. A few hours later the animal would be deeply comatose and very cold, with respirations separated by long intervals, and a very slow, weak heart-beat. It was our custom to kill the animal as soon as these terminal symptoms made their appearance. We thus obtained emulsions of amoebae in a much more healthy and active condition than those taken from the animal even a few hours after the circulation had stopped.

A post-mortem examination was made on every infected kitten. With the exception of the hepatic abscess already mentioned we found nothing of importance in organs other than the intestine. The lymphatic glands draining the large intestines were almost always enlarged, and often contained pus. No amoebae, however, were discoverable in this pus, or in sections of the glands. Death was presumably due to septic absorption from the intestine; though, since no blood-cultures were made, we are not in a position to state whether septicaemia occurred, or whether the absorption of toxic substances was sufficient to account for death. In most instances the blood in the vessels had a notably bright, arterial hue, so that the viscera had an appearance recalling that seen in death from poisoning by carbon monoxide.

The ulceration of the mucous membrane of the large intestine was in almost all cases extensive, but showed many variations. The portions most severely affected were, as a rule, the upper and lower thirds of the large intestine. In the lower third the mucous membrane was often very deeply ulcerated. The muscular coat and submucous tissue in such cases showed much inflammatory thickening. Abscesses were found in the submucous tissue, the creamy pus from which was crowded with active

amoebae.   The ulceration in this region in a few cases involved the muscular coat, and in one instance perforated it.   The ulcers in the upper third, including the caecum, were usually more superficial, with a clearly marked hyperaemic edge.   Frequently almost the whole of this region was eroded by coalescent shallow ulcers, involving also the lips of the ileo-colic sphincter.   The middle third of the large intestine was commonly less affected, though scattered ulcers usually occurred here also.   In a few cases nearly the whole mucous membrane of the large bowel was superficially eroded by innumerable small ulcers.   This condition was associated with a longer duration of the disease and the passage of relatively little blood.

The limitation of the infection to the large intestine was in most cases very sharp, the ulceration in only two cases spreading on to the mucous membrane of the ileum.   In these it extended for about 1 cm. above the sphincter, a stricture marking externally the abrupt ending of the ulceration.   In kittens which had lain dead for some hours, amoebae were several times found in the lower ileum.   We concluded, however, that they had wandered above the sphincter after the death of the host.

B. *The strain obtained by infecting with cysts from man*

Our attempts to infect kittens per os with cysts from human cases began with a series of 7 failures.   Each kitten was given by stomach-tube about 5 cc. of faecal emulsion containing many healthy-looking, ripe cysts of Entamoeba histolytica from three human carriers.   One kitten died 9 days after receiving the cysts, the post-mortem examination showing an inflamed condition of the bowel, possibly due to bacterial infection, but no sign of ulceration or amoebae.   Two other kittens passed small quantities of blood and mucus in the faeces intermittently for a week or two following the administration of the cysts.   These kittens were killed for examination, and in each a few minute hyperaemic patches were found on the mucous membrane of the large bowel, but no ulceration and no amoebae.   The remaining 4 kittens were examined daily for about a month, with consist-

ently negative results; two of them receiving a second dose of cysts by the mouth from a second case, with similar results. Finally all four received injections per anum of active amoebae of strain A from other kittens; and all then acquired a typical and fatal dysentery.

Some months later a further and successful attempt was made with cysts from the faeces of another human carrier. The history of the case has points of interest.

The case was no. XVI of a series published elsewhere by one of us (C. D., 31). He contracted dysentery in Gallipoli in 1915, and was treated in Egypt with emetine hydrochloride hypodermically. The details of treatment are not available. He was discharged from hospital as cured and sent to England, where further examination revealed a chronic infection with Entamoeba histolytica, for which emetine hydrochloride was again given. The amount given is not known, but according to the patient's own statements he received a large number of injections. He was again discharged as cured, but when subsequently examined by one of us was found to be still passing cysts of Entamoeba histolytica. He was then given yet another course of hypodermic injections of emetine hydrochloride (1 grain daily for 12 consecutive days). The cysts disappeared from the faeces during and immediately after treatment, which finished on May 8th, but were present again on May 26 and subsequently. On June 26 a specimen of faeces was emulsified and given by the stomach-tube to two kittens. On July 8 the patient began a 12-day course of the double iodide of emetine and bismuth. He had ceased passing cysts of Entamoeba histolytica on July 12, and since then has been apparently free from infection.

The material given to the kittens had been passed 3 days previously and kept in a corked bottle at the ordinary summer temperature of the laboratory. One kitten died between the 11th and 12th days after receiving the cysts. An examination could not be made before the mucous membrane was much disintegrated by post-mortem change, and nothing of significance was found. On the 12th day the other kitten, which had passed

loose faeces for some days previously, passed some blood-stained mucus containing active amoebae. On the 13th and 14th days saline washings from the bowel were used to infect another kitten, and on the 15th day the kitten was killed, though still vigorous, and the emulsion from its large intestine used to infect a third kitten. From these the strain was then passed in the usual way. At the 7th attempted passage the strain was lost, the kitten reserved as a source of infective material having yielded an unexpectedly poor emulsion of amoebae. Only 12 kittens were infected with the strain, the incubation periods, excluding that of the first infection with cysts, being as follows:

Incubation period	Number of cases
2 days	2
3 days	2
4 days	5
5 days	1
6 days	1
	—
	11

Average incubation period: a little under 4 days.

The average incubation period is, therefore, nearly twice as long as with strain A; and though we are dealing with only a small number of infections with strain B, the occurrence of a 4-days incubation in almost one-half of the number seems to indicate that the difference is a real one. This is corroborated by the duration of the infections. Some of the cats were killed while still quite vigorous, and others were experimentally treated; but after eliminating these, we get the following figures:

Duration of infection	Number of cases
5 days	1
6 days	1
7 days	1
8 days	2
10 days	1
11 days	1
24 days	1
	—
	8

Average duration: 10 days.

Such an average has probably little significance, but the fact that the majority of cases lasted more than 7, and one case as long as 24 days, seems to indicate a less rapid progress of this infection than of that with strain A. In the cases of longer course, the slower advance of the infection was indicated by the fact that ordinary faecal material was passed for several days after the infection was established. The impression that the strain had a lower virulence than strain A, and less tendency to invade the tissues, was confirmed by post-mortem examination. As a rule the mucous membrane of the large intestine was uniformly sprinkled with minute erosions and hyperaemic spots, and was coated with a tenacious mucus crowded with amoebae. In the more rapidly fatal cases, however, the clinical course and the post-mortem findings resembled those of strain A. In one case, which ended fatally in 5 days, the ulceration was extensive and deep, the submucosa being greatly thickened and containing amoebic abscesses which at several points had burrowed through the muscular coats to the peritoneum; in fact the invasion of the tissue was as severe as in any case infected with strain A. The difference in virulence was, therefore, not consistently so great as it might have appeared if only a few infections with each strain had been studied.

Attempts to infect other animals

We made two unsuccessful attempts to transmit our strain A from kittens to monkeys (Macacus rhesus). We also tried without success to infect rats and rabbits—the latter by injection of amoebae into the small intestine under anaesthesia.

Of two puppies injected per anum with the same strain, one acquired a mild infection and passed mucus with a little blood for a week, active amoebae being found on three occasions. The infection then disappeared spontaneously. No amoebae or cysts were subsequently discoverable, and the intestine showed no lesion when the animal was killed and dissected.

Walker and Sellards (9) likewise failed, after repeated attempts, to infect monkeys with Entamoeba histolytica. The occurrence of spontaneous amoebic dysentery has, however,

been several times recorded in these animals. Lösch (1) and Hlava (2) succeeded in infecting dogs, and spontaneous outbreaks of amoebic dysentery in these animals are also on record. Lynch (10) claims to have transmitted Entamoeba histolytica from man to the rat and to have observed a naturally occurring amoebic dysentery in this animal.

IV. THE EFFECT ON ENTAMOEBA HISTOLYTICA IN VITRO OF VARIOUS ALKALOIDS AND OTHER SUBSTANCES

Methods of observation

The experiments, with one exception mentioned later, were made on amoebae obtained from the infected kittens. A few early experiments were made by the method used by Rogers (11), of placing on a slide a droplet of the slime containing active amoebae, adding a few drops of a warm solution of the required composition and concentration, covering with a coverslip and then watching the result under the microscope with a warm stage. We soon abandoned this, as we found that the results of the observations were not consistent. The superficial slime from the infected intestine contains amoebae in all stages of vitality, from those which are obviously dead to those which are healthy and vigorously motile. By "obviously dead" we do not, of course, mean merely rounded and motionless, but swollen by imbibition, with clearly visible nuclei. The proportion of healthy amoebae varies widely in different droplets of such mucus; it is difficult to guarantee their thorough admixture with the solution; and it is impossible to decide whether the dead amoebae found have been killed by the solution, or were dead beforehand.

We early discarded this method, therefore, in favor of thoroughly mixed emulsions in saline. The preparation of these has already been described. It need only be noted here that thoroughly scraped mucous membrane yields a far higher proportion of healthy amoebae than mucus passed per anum; and that the mechanical violence entailed in mixing the emulsion probably disintegrated many of the dead and less resistant amoebae, so that from a freshly killed kitten we usually obtained an emulsion in which the vast majority were thoroughly healthy.

The emulsion was poured into a test-tube and kept in a thermostat at 37° C. for about 15 minutes. If the amoebae were then healthy and active, a series of samples was measured into other tubes, and measured volumes of solutions in physiological saline of the drug to be tested were added, with rapid mixing, so as to make the series of dilutions in which the action was to be tested. The tubes, with the untreated residue of emulsion as a control, were then returned to the thermostat. At intervals a well mixed sample was withdrawn from each by a capillary pipette, placed on a warmed slide, covered with a coverslip supported with droplets of wax, and immediately examined under the microscope. (We generally used a 4 mm. apochromat objective, with compensating ocular 4.) In the earlier experiments we used a warm stage, but later came to the conclusion that this was unnecessary. In a drop of the solution placed on a warmed slide, the surviving amoebae retained their activity without perceptible impairment during the few minutes needed for each observation.

The action of the alkaloids of ipecacuanha and of some derivatives

The introduction during recent years of salts of emetine in place of ipecacuanha, for the treatment of amoebic dyentery, was based on observations of the effect on amoebae in vitro of solutions of pure emetine salts, as contrasted with that of extracts of ipecacuanha deprived of its alkaloids. The first experiments of this kind were made by Vedder (12) in 1912, who used cultures of free-living amoebae. He found that such amoebae were killed by emetine hydrochloride and cephaeline in a dilution of 1 in 100,000, while extracts of ipecacuanha freed from alkaloids had but little amoebicidal activity. Rogers (11) made similar observations on Entamoeba histolytica in the stool of a sufferer from acute dysentery. He found that 1 in 10,000 emetine hydrochloride killed the amoebae immediately, while even in a solution containing 1 part in 100,000, they became rounded up and degenerated within a few minutes. He found cephaeline to have about the same amoebicidal action as emetine, but a higher toxicity for man. Kuenen and Swellen-

grebel (13) tested the action of emetine hydrochloride on active Entamoeba histolytica, and likewise on its cysts, also using material from human cases. Their test of death of the organism was, in all cases, the power of eosin to stain the protoplasm, not cessation of movement. In a first experiment with "histolytica" amoebae, 1 in 10,000 emetine killed within an hour, when the specimen was fresh; in faeces examined 7 hours after being passed, the same dilution killed all in a few minutes. In a second experiment death of all the amoebae "in a few minutes" was again observed with 1 in 10,000 emetine. There is here a considerable reduction of the speed of action of emetine on freshly passed amoebae from that found by Rogers; there is also evidence of a weakening of their resistance to emetine after they have passed from the bowel. The "minuta" forms appeared to be more resistant. In a first experiment they were still partly motile after 45 minutes in emetine 1 in 10,000; and even after four hours some were still unstainable with oesin. In a second experiment some were still motile after 4 hours in 1 in 5,000 emetine; and even after 48 hours some unstainable amoebae were found. Experiments were also made with encysted forms, the results being recorded statistically by counting the proportion of stained to unstained cysts. It is difficult to attach significance to these results, since there is no apparent correlation between the killing power of emetine and its concentration. Thus, in one instance, the percentages of dead cysts in preparations treated for half an hour with emetine 1 in 100, 1 in 1,000, and 1 in 10,000 respectively, works out at 77.2, 61.8, and 74. One may therefore doubt whether emetine had any effect at all on the cysts.

In our experiments we examined the action, on Entamoeba histolytica, not only of the three known alkaloids of ipecacuanha, but also of a fourth, which had not then been described, and of several new artificial derivatives. This extension of our work was made possible by the kindness of Dr. F. L. Pyman, Director of the Wellcome Chemical Research Laboratories, who liberally placed at our disposal supplies of pure salts of the alkaloids and their derivatives, all prepared by himself, and several new to science. We are further indebted to Dr. Pyman for assistance in drawing up the following note on the compounds tested.

The three alkaloids of ipecacuanha hitherto recognized—emetine, cephaeline, and psychotrine—are built up on a common structural plan, of which little is yet known beyond the fact that it includes an isoquinoline nucleus. Psychotrine contains a phenolic hydroxyl and also an unsaturated linkage; when the latter is saturated by introduction of two hydrogen atoms cephaeline results, together with isocephaeline, which is a stereo-isomer. Cephaeline is, therefore, also a phenolic base. If the hydrogen of the phenolic hydroxyl of cephaeline is replaced by CH_3, emetine is produced.

It will be clear that, if the phenolic hydroxyl of psychotrine were similarly methylated, a base methyl-psychotrine should result, which, on reduction of the unsaturated linkage, should yield emetine and an iso-emetine. Pyman (14) has demonstrated the production of all these substances from psychotrine by the changes indicated, and has further succeeded in isolating methyl-psychotrine from ipecacuanha residues, in which it occurs in a small proportion (about 0.03 per cent of the root). We have therefore, to start with, four alkaloids naturally occurring in ipecacuanha[3]—cephaeline, emetine, psychotrine and methyl-psychotrine and may represent their relationship as follows:

$$\text{Psychotrine} + H_2 = \text{Cephaeline}$$
$$\text{Methyl-psychotrine} + H_2 = \text{Emetine}$$
$$\text{Psychotrine} - H + \text{Me} = \text{Methyl-psychotrine}$$
$$\text{Cephaeline} - H + \text{Me} = \text{Emetine}$$
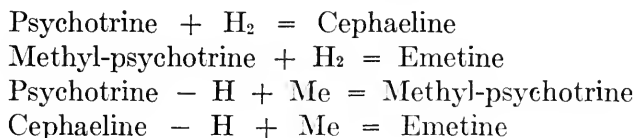
It will be evident that if the hydrogen of the phenolic hydroxyl of cephaeline were replaced by other alkyl radicles in place of methyl, a series of homologues of emetine could be produced. Some of these were described, while our work was in progress, by Karrer (15), and a whole series of them has been patented by Meader (16). Dr. Pyman kindly made us a sample of ethyl-cephaeline, which we tested in our series, From the products of the reduction of methyl-psychotrine, in addition to emetine

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[3] Since our experiments were made Pyman has described yet another alkaloid occurring in small traces in ipecacuanha. He has called it "emetamine," but its relation to the other alkaloids is as yet unknown.

and isoemetine, a well crystallized base giving a crystalline sulphate was isolated by Pyman (14).   On investigation it was found that the treatment had replaced one of the methoxy-groupings in methyl-psychotrine by an atom of hydrogen.   Probably the unsaturated linkage was reduced at the same time, so that the new substance is probably demethoxyemetime, or demethoxy-isoemetine.   In his recent paper Pyman, in view of the uncertainty as to its exact structure and relation to the other alkaloids, has called it "Base C."   Here we have referred to it as "demethoxy-emetine," a name indicating its probable relations with sufficient accuracy for our purpose.   Derivatives of yet another type have been obtained by Carr and Pyman (17) from emetine and cephaeline, by a process which leads to the replacement of the hydrogen atom of the imino-grouping by a methyl-radicle, thus producing N. methylemetine and N. methyl-cephaeline. One of us (H. H. D.) had the opportunity of examining these derivatives some years ago, and found them much less toxic than emetine or cephaeline.   It seemed desirable to test their action on Entamoeba histolytica, and N. methyl-emetine was accordingly included in our series.   The action of several of these substances on cultures of free-living amoebae, together with that of some other derivatives which we did not test, was studied a few years ago by Dr. (now Lieutenant-Colonel) C. M. Wenyon, who has recently published the results in a joint paper with Dr. Pyman (18).

The preparations at our disposal for the tests were emetine hydrochloride, cephaeline (alkaloid), methyl-psychotrine sulphate, psychotrine (alkaloid), ethyl-cephaeline hydrobromide, "demethoxy-emetine" sulphate, N. methyl-emetine sulphate. The salts were made up in 1 per cent solution in the physiological saline used for the emulsions of amoebae.   Those alkaloids which were only available as the free base were weighed as such and rubbed up in saline with the addition of a minimal excess of hydrochloric acid, so as to make a strong solution of the hydrochloride; the solution was then neutralized with dilute soda solution, and diluted to 1 per cent of the alkaloid.   The different 1 per cent solutions are, therefore, not exactly equivalent in mo-

lecular concentration.   There is, however, a more serious discrepancy in that the different salts and alkaloids crystallize with a varying number of molecules of water of crystallization.[4]   We have not thought it worth while to introduce corrections for these differences.   The expression of dilutions in molecular equivalents would give the results a superficial appearance of greater·scientific accuracy; we found, however, that the error due to variations in the resistance of different emulsions of amoebae was of a range altogether outside that introduced by weighing one alkaloid. as base, another as sulphate, and ignoring the presence of water of crystallization.   Even in the same emulsion it was evident that individual amoebae differed widely in their resistance, so that an exact determination of lethal concentration was out of the question.   The kind of result which we usually obtained is exemplified in the three following experiments. A finer discrimination was attempted when it seemed practicable, and an effort was made to reduce the observations to a common standard by testing the effect of each of the other alkaloids in parallel with that of emetine, using the same emulsion for both and putting up the dilutions simultaneously.

May 25, 1916.   Emulsion from kitten 5, strain A.   Tested with emetine hydrochloride in dilutions as follows.   (The times indicated are the periods after making the mixture at which specimens were removed for examination.)

Experiment 1:
　1 in 100,000 and 1 in 10,000.   45 minutes.   All amoebae living and
　　　　healthy; indistinguishable from the control.
　1 in 1,000.   Nearly all dead in 5 minutes, all dead in 15 minutes.
Experiment 2:
　1 in 100,000 and 1 in 10,000.   As good as the control after 20 minutes.
　1 in 1,000.   No living amoebae found after 5 minutes.

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[4] Dr. Pyman kindly informs us that the following percentages of water of crystallization occurred in the preparations supplied to us: Cephaeline 5.5 per cent; psychotrine 13.4 per cent; methyl-psychotrine sulphate 17.9 per cent; ethyleephaeline hydrobromide 23.2 per cent; demethoxyemetine sulphate 3.2 per cent; N. methyl-emetine sulphate 8.4 per cent.

Experiment 3:

    1 in 100,000 and 1 in 10,000.   As good as the control after 90 minutes.

    1 in 1,000.   Nearly all dead in 10 minutes, but a careful search after 90 minutes revealed a few just moving.

These preliminary trials gave promise of a clear cut determination of the lethal concentration of emetine, which later experience unfortunately did not altogether fulfil.   Already, it will be noted, there was a marked discrepancy between our experience and that of Rogers, who observed a rapidly lethal effect even with 1 part of emetine hydrochloride in 100,000.

May 28.   Emulsion from kitten 31, strain A.   A very good emulsion, of uniformly healthy and active amoebae, which enabled observations to be prolonged for 3 hours.   Tested with emetine hydrochloride.

    1 in 100,000.   A few amoebae dead at the end of 3 hours, but the remainder as active as the control.   A doubtful and negligible effect.

    1 in 10,000.   1 hour, all living; 3 hours, a fair number dead, but the rest active.

    1 in 1,000.   25 minutes, most dead, but some healthy and active; 3 hours, a few healthy survivors.

June 28.   Emulsion from kitten 50, strain A.   Tested with N. methyl-emetine sulphate.

    1 in 100,000.   50 minutes.   A few amoebae dead, but the rest equally active with the control.   Doubtful if any action.

    1 in 10,000.   40 minutes.   A few show crawling movement, and others spasmodic extrusion of pseudopodia, without progression; the majority already dead.

    1 in 1,000.   30 minutes.   Nearly all dead, but some show spasmodic movement, with relapse into the spherical condition, and no change of position.   (In later observations we frequently observed the termination of this type of movement in bursting and disintegration of the amoeba.)

    1 in 2,000 and 1 in 5,000.   After the same period showed a similar condition, but with decrease of the proportion already dead, and increase of those showing the ineffective changes of shape.   None crawling effectively were found in either of these dilutions.

In the control activity was well maintained 2 hours after preparation.

July 24. Emulsion from kitten 68, strain B. (This is of interest as being the only complete and satisfactory test of emetine on the amoebae of strain B. in vitro.) Tested with emetine hydrochloride.

1 in 100,000. 30 minutes. All living and crawling vigorously.
1 in 10,000. 30 minutes. All living and crawling vigorously.
1 in 1,000. 30 minutes. All living and crawling vigorously.
1 in 100. 30 minutes. All living and crawling vigorously.

Even in the 1 per cent dilution, which was made by mixing equal volumes of the emulsion and of a 2 per cent solution of emetine hydrochloride in saline, the amoebae showed no sign of injury, being indistinguishable from the control emulsion at the end of half an hour.

September 6. Emulsion from kitten 98, strain B. Although the kitten was killed for this experiment while still in good condition, the emulsion obtained was not suitable for the test, the amoebae, though abundant and actively motile, being vacuolated and degenerate. In view of the high resistance shown by this strain in the previous test, a sample was put up with emetine hydrochloride 1 in 1,000, and not examined till it had been for 45 minutes in the thermostat. It was then found that all the amoebae were dead; but examination of the control (untreated emulsion) showed that a large proportion of the amoebae had died in this also, though some were still living. Evidently emetine 1 in 1,000 had a perceptibly injurious action on the unhealthy amoebae. No further tests were possible; and the imperfect experiment is quoted to illustrate the wide variations of result obtainable with different specimens of the same strain of amoebae.

September 21. Emulsion from kitten 113, strain A. A preliminary test of the effect of methyl-psychotrine sulphate.

1 in 10,000. 30 minutes. The large majority dead; a few still active.
1 in 1,000. 30 minutes. Nearly all dead; a few just moving.

September 28. Emulsion from kitten 117, strain A. A comparative test of the effects of emetine hydrochloride, methyl-psychotrine sulphate, and psychotrine (as hydrochloride).

1 in 1,000. Emetine. 30 minutes. Most amoebae still alive, but sluggish.
      90 minutes. Practically all dead, a few showing very sluggish movement.
    Methyl-psychotrine. 30 minutes. Most rounded and motionless, a few normal.
      60 minutes. Most rounded and motionless, a few normal.
      90 minutes. Practically all dead, a few moving feebly.

Psychotrine. 30 minutes. Normal; indistinguishable from control.

60 minutes. Normal; indistinguishable from control.

90 minutes. Normal; indistinguishable from control.

1 in 10,000. None of the three alkaloids had any perceptible effect in 90 minutes. The effect of methyl-psychotrine was thus not materially different from that of emetine, while psychotrine was without action in the dilutions used.

October 2. Emulsion from kitten 120, strain A. A comparative test of emetine hydrochloride and cephaeline (as hydrochloride).

1 in 1,000. Both alkaloids killed nearly all the amoebae within an hour; most had disintegrated and disappeared, and the few remaining exhibited only spasmodic and ineffectual protrusion of pseudopodia, which could occasionally be seen to end in bursting and dissolution.

1 in 10,000. Neither had any distinct action in an hour, the solutions after that period containing abundant, actively crawling amoebae; neither a greater proportion of dead amoebae, nor any reduction in the richness of the emulsion could be detected in either, as compared with the untreated control.

October 3. Emulsion from kitten 119. (This kitten had been treated, without effect, by daily washing out the large intestine with emetine 1 in 10,000.) A comparison of the effects of emetine hydrochloride and cephaeline (as hydrochloride).

1 in 1,000. Emetine. 20 minutes. Nearly all amoebae broken up and disappeared; the few still visible are dead.

60 minutes. The same.

Cephaeline. 25 minutes. Nearly all broken up and disappeared, but, of the few remaining intact, some show feeble movement.

60 minutes. A few still show weak, spasmodic movement.

1 in 10,000. Emetine. 30 minutes. Most dead, but of the remainder some are still crawling.

60 minutes. A few still living.

Cephaeline. 35 minutes. Most dead, some moving but none crawling.

60 minutes. Most disintegrated; none found living.

Control. 60 minutes. Most of the amoebae are motionless, but a few still crawling actively.

Neither in this, nor in the previous experiment can any difference

be detected between the action of cephaeline and that of emetine. The experiment of October 3 shows that the amoebae surviving the daily irrigation with emetine (1 in 10,000) are more, rather than less liable to the injurious effect of emetine or cephaeline in vitro than the amoebae from an untreated infection with this strain. This greater liability, however, is evidently, as in other cases, only an expression of generally impaired resistance, since the amoebae in the control emulsion died off with abnormal rapidity in this case.

October 24. Emulsion from kitten 136, infected with an emulsion of strain A, which had been treated for 45 minutes with 1 in 10,000 emetine hydrochloride in vitro, and then washed before injection. Tested with emetine hydrochloride.

1 in 10,000. No effect in 1 hour; apparently quite as active as control.

1 in 1,000. No effect in 1 hour; apparently quite as active as control.

1 in 200. Nearly all dead in 30 minutes. All dead in 1 hour.

This result suggested an increase of resistance of strain A to emetine, as a consequence of the treatment of the infecting emulsion with emetine. The suggestion was probably incorrect, since emulsions of the untreated strain from later passages showed a similar resistance. (See the next experiment, November 15).

November 15. Emulsion from kitten 145, strain A. Comparative test of emetine hydrochloride and demethoxy-emetine sulphate.

1 in 10,000. Emetine. $1\frac{3}{4}$ hours. Normal and indistinguishable from the control.

Demethoxy-emetine. $1\frac{3}{4}$ hours. Normal and indistinguishable from the control.

1 in 1,000. Emetine. 20 minutes. Normal and indistinguishable from the control.

60 minutes. Normal and indistinguishable from the control.

115 minutes. Normal and indistinguishable from the control.

Demethoxy-emetine. 25 minutes. Normal and indistinguishable from the control.

65 minutes. A few dead, but most quite normal.

115 minutes. Some dead, with clearly visible nuclei; others normal.

1 in 500. Demethoxy-emetine. 20 minutes. All dead and many disintegrated.

1 in 200. Emetine. 10 minutes. Many dead, some still moving.

35 minutes. Most dead, a few still moving.

55 minutes. All dead and disintegrating.

Demethoxy-emetine. 15 minutes. All dead and many disinte-
grated.
35 minutes. Most have disappeared; the few remaining are
dead.

It will be seen that, while neither has any effect in 1 in 10,000 dilu-
tion, the superior activity of demethoxy-emetine is visible when the
concentration is raised to 1 in 1,000, in which strength this derivative
shows a distinct action, while emetine itself was still without action on
this emulsion in the time for which observation was continued. In 1
in 500 dilution demethoxy-emetine is more rapidly lethal for the amoe-
bae than emetine 1 in 200.   This relation of activities is confirmed in
the experiment of November 22 (infra).

November 21. Emulsion from kitten 149, strain A. Comparative
test of emetine hydrochloride and ethyl-cephaeline hydrobromide.

1 in 10,000. Emetine. 40 minutes. Most are still crawling actively,
but some have disintegrated, only faint outlines or
"shadows" remaining.
Ethyl-cephaeline. 35 minutes. Not distinguishable from the
above.
Control. 40 minutes. All living and crawling actively; no
"shadows" seen.
1 in 1,000. Emetine. 20 minutes. Many disintegrated; re-
mainder mostly sluggish, but some crawling.
70 minutes. Most have disappeared; the few still visible
making feeble, ineffective movements.
Ethyl-cephaeline. 25 minutes. Most disintegrated, the remain-
der swollen, and making convulsive ineffectual move-
ments.
65 minutes. Only a few remain, making feeble ineffective
movements.

There is evidently but little difference in activity between the two
substances, but the result is slightly in favor of ethyl-cephaeline.

November 22. Emulsion from kitten 150, strain A. Comparative
test of emetine hydrochloride, ethyl-cephaeline hydrobromide, and
demethoxy-emetine sulphate.

1 in 10,000. The preparations remained normal and indistinguish-
able from the control with all three substances in this
dilution, as long as observation was continued (40 minutes).
1 in 1,000. Emetine. Normal as long as observation was con-
tinued (90 minutes).

Ethyl-cephaeline.   15 minutes.   Normal.

    35 minutes.   Most living, but comparatively sluggish.

Demethoxy-emetine.   15 minutes.   Most dead, a few moving.

    30 minutes.   Nearly all dead, but 2 found crawling.

1 in 500.   Emetine.   30 minutes.   A few dead but many crawling normally.

Ethyl-cephaeline.   30 minutes.   Many dead, some changing shape, but none crawling.

Demethoxy-emetine.   20 minutes.   All certainly dead.

1 in 200.   Emetine.   10 minutes.   Most dead, some changing shape, but none crawling.

    20 minutes.   All dead and disintegrated.

Ethyl-cephaeline.   10 minutes.   Most dead, some changing shape, one found crawling.

    20 minutes.   Most disappeared, many breaking up, one found crawling feebly.

The experiment confirms the superior lethal effect of demethoxy-emetine, as compared with the other two.   There is again no clear distinction between the activity of emetine and of ethyl-cephaeline; the latter appears to be more active in 1 in 500 dilution, while the former is the more completely lethal in a 1 in 200 dilution.   Possibly this last apparent difference is due to the inclusion of a few more resistant amoebae in the sample put up with ethyl-cephaeline; but in any case the order of activity is the same in the two cases.

November 26.   Emulsion from kitten 152, strain A.   Comparative test of emetine, methyl-psychotrine, and N. methyl-emetine. •

1 in 10,000.   None of the substances had any perceptible effect in an hour, the three preparations being indistinguishable from the control at the end of that period.

1 in 1,000.   In the case of all three a good many amoebae were dead at the end of 30 minutes, but all the preparations still showed a fair number still normal and crawling actively.

1 in 200.   Emetine.   30 minutes.   Many dead, but a good number still walking actively.

Methyl-psychotrine.   30 minutes.   Most dead; a few show weak streaming movements.

N. methyl-emetine.   30 minutes.   Indistinguishable from the preparation with methyl-psychotrine.

An inspection of these results will make clear the range of variation in resistance exhibited by different samples even of the same strain of

amoebae, obtained from animals similarly infected and at approximately the same stage of infection. This variation makes it impossible to assign a definite lethal concentration for Entamoeba histolytica to any of these alkaloids. It also makes comparison of their activities impossible, except when the different · alkaloids were tested simultaneously on the same emulsion of amoebae. Though the results obtained do not permit of precise numerical expression, the substances can be arranged roughly in order of their activities. The activities of emetine and cephaeline appeared to be identical, while psychotrine was not perceptibly active in the concentrations tested. Ethyl-cephaeline appeared to be slightly, but not very certainly, more active than emetine; methyl-psychotrine and N. methyl-emetine were distinctly more active than emetine, though the difference was not great; while demethoxy-emetine was decidedly the most active of all. The order of activity appears, therefore, to be as follows:

Demethoxy-emetine > Methyl-psychotrine = N. methyl-emetine > Ethyl-cephaeline ≧ Emetine = Cephaeline > Psychotrine.

The question whether these results have any therapeutic implication will be considered later. At present the point of greatest interest is the contrast between our own results as to the concentration of emetine which kills the amoebae *in vitro*, and those obtained by earlier observers such as Rogers. It will be remembered that Rogers found that 1 in 100,000 emetine hydrochloride caused immobilization and degeneration of the amoebae within a few minutes. In contrast with this, we failed so completely to detect any influence of emetine in this dilution, unless the examination were continued to the limit of survival of the control, that we did not trouble to test its action in a large proportion of the experiments. Even with the 1 in 10,000 dilution, which Rogers (11) found immediately lethal, it was exceptional for any action to be perceptible within the period of 30 to 60 minutes for which observation was usually continued. So rarely did we observe any definitely greater rate of mortality of amoebae in this dilution than in the control saline, that we do not hesitate to conclude that, on healthy and otherwise uninjured amoebae of the two strains from our kittens, 1 in 10,000 emetine hydrochloride has no perceptible action in vitro. Many of the emulsions showed a much higher resistance than this. In

one experiment on strain B even 1 per cent emetine had no perceptible action in 30 minutes; while, in the last experiment recorded, on an emulsion of strain A, it will be seen that a fair proportion of the amoebae resisted 1 in 200 emetine for the same period. Several explanations of the discrepancy between Rogers' results and ours at once suggest themselves.

1. We were inclined to believe at first that our results were explicable by an acquired resistance of these strains of amoebae to emetine. Both were obtained from patients who had received several courses of treatment with hypodermic injections of emetine, and in one case with ipecacuanha as well, without being cured. The development of resistance to the drug under such conditions would be in accord with the demonstrated action of other drugs on other protozoa. Such a conception, however, received a convincing disproof in the later history of the patient furnishing strain B. This patient was unsuccessfully treated with emetine, in considerable quantities, before our kittens were infected from his cysts (*vid. sup.*). He was then given, at our suggestion, a course of the double iodide of emetine and bismuth by the mouth (36 grains, corresponding to rather more than 12 grains of emetine hydrochloride, in 12 days). The result was a prompt and apparently permanent disappearance of the infection, no cysts or amoebae being discoverable in his faeces in 14 examinations made over a period of 215 days following treatment. The cure of this patient seems to be as thoroughly established as any in the series of cases which one of us (C.D., 31) has examined and elsewhere recorded. Yet with the cysts from his faeces we established in kittens a strain of amoeba which, in vitro, seemed unaffected even by 1 per cent emetine.

2. It might be suggested that our observation of the amoebae under the microscope failed to detect the effect of emetine. This seemed to us unlikely; in any case changes of the gross and obvious type described by Rogers could hardly be missed, and their occurrence would be entirely incompatible with our observation of amoebae crawling vigorously across the field of the microscope for an hour or more in the emetine solutions. However, in order to exclude such a suggestion, we decided to

test the power of the emulsions, after incubation with emetine, to infect fresh kittens. This experiment also served to test the further possibility that the emetine, though without visible action on the amoebae, might impair their power of multiplication, and hence annul their infectivity. The following are records of such experiments.

October 12. Emulsion from kitten 127, strain A. Kittens 130 and 131 each received 8 cc. at once per rectum. Both acquired typical, fatal infections, beginning on October 14 and 15 respectively. With 9 cc. of the emulsion, 1 cc. of 1 in 1,000 emetine hydrochloride was mixed, making a dilution of 1 in 10,000. After 45 minutes incubation the majority of the amoebae were vigorously active. 9 cc. of the treated emulsion were then injected per rectum into kitten 132. This remained well until October 17. On this day a fresh emulsion was made from kitten 130, and 20 cc. of this were made up similarly to contain 1 in 10,000 emetine, and incubated for an hour. The 20 cc. were then divided into two lots of 10 cc., of which one was used for a second rectal injection into kitten 132. From the other portion of 10 cc. the amoebae were thrown down by centrifuging for a few minutes at low speed. The supernatant fluid, containing emetine, was sucked off and replaced by clean saline; the process was repeated, and the amoebae emulsified in a second quantity of saline. This emulsion was then injected into a new kitten, no. 136. Of the remainder of the emulsion, which had not been treated with emetine, 8 cc. were injected into another new kitten, no. 135. We thus had 3 kittens under observation from October 17.

No. 135, which had received a normal injection of untreated amoebae.

No. 132, which had received two injections of amoebae incubated with 1 in 10,000 emetine, and injected in the solution containing the alkaloid in this proportion.

No. 136, which had received an injection of amoebae similarly treated with emetine, but washed and suspended in clean saline for injection.

No. 135, first passed blood, mucus and amoebae on October 19 and died on October 20.

No. 132, remained normal until October 20, when it passed loose faeces and vomited. Unfortunately it died during the evening of that day, and was not dissected till next morning. The mucous membrane of the lower portion of the

large intestine showed numerous small ulcers of the usual amoebic type. In scrapings from these amoebae were discovered, many containing red corpuscles, and some still exhibiting a feeble motility.

No. 136, remained well till October 20, when it passed a small quantity of blood-stained mucus containing numerous active amoebae. It continued to pass such material in increasing quantity till October 24, when it was killed. The usual extensive ulceration of the mucous membrane of the large intestine was found post-mortem. An emulsion of the amoebae from the intestine was prepared and tested with emetine in vitro, as described in the experiment under the date of October 24.

November 23. Emulsion from kitten 151, strain A., which was killed before the onset of terminal symptoms. 8 cc. injected at once into a new kitten 152. Of the rest 19 cc. were taken and 1 cc. of 2 per cent emetine was added, making a concentration of 1 in 1,000 emetine. The mixture was incubated for 30 minutes. It still contained a number of healthy-looking, active amoebae. The whole was centrifuged, and the deposit washed with saline, and made up with fresh saline to 6 cc. This was injected into a new kitten, no. 153.

Kitten 152 began to pass blood mucus and amoebae on the following day, November 24, and was killed on November 26. The mucous membrane of the large intestine showed a large number of small, superficial ulcers, and was covered by a tenacious mucus containing large numbers of active amoebae.

Kitten 153 remained quite well until November 29, when it began to pass thin, bloodless mucus, full of active amoebae. It was found dead on December 1. On dissection practically the whole of the mucous membrane of the large intestine was found to be superficially ulcerated. The abundant mucus covering it was crowded with amoebae; unfortunately, the kitten having been dead for many hours, the amoebae were not in sufficiently good condition for a test of their resistance to emetine.

Against these successful infections with amoebae thus treated with emetine must be placed four other similar experiments in which the amoebae, after similar exposure to 1 in 10,000 emetine, failed to infect. In two of these cases the emulsion was injected directly, together with the added emetine; in the other two the amoebae were washed after treatment, and injected in fresh saline. Putting together the attempts

at infection with amoebae treated with emetine 1 in 10,000, we have 1 success out of 4 attempts in which the emetine was injected with the amoebae, and 1 success out of 3 in which the emetine solution was replaced by clean saline for injection. In the one experiment in which the amoebae were treated for 30 minutes with 1 in 1,000 emetine and then washed, the infection was successful. While, therefore, the survival and infectivity of amoebae in such concentrations of emetine, for periods up to an hour, are demonstrated, the infection obtained with them after such treatment is less regular, and the prolongation of the incubation period and of the interval between onset and death is more evident, than we should have expected from the proportion of microscopically normal amoebae seen in the preparations after such treatment.

3. Our observations are the first in which the action of emetine and other substances has been tested in vitro on Entamoeba histolytica obtained from kittens, previous workers having used material from human infections. It seemed possible, therefore, that this might explain the difference between our results and theirs. It was conceivable that the cat, as host, might so modify the amoebae as to raise their resistance to emetine; though it should be noted that the increase of resistance would have to be enormous to explain the discrepancy between our results and those of Rogers (11). The possibility could only be tested by repeating our observations on amoebae from a human case. After several vain attempts to obtain suitable material we at length succeeded through the kind coöperation of Dr. Helen Chambers. The patient was a soldier who had never left England until he was sent to the front in France, whence he was invalided home, suffering from "trench-feet" and "diarrhoea." It is very improbable that he had received any emetine before we procured the specimen. After admission to the Women's Military Hospital, Endell Street, he began to pass blood and mucus, in which Dr. Chambers discovered abundant Entamoeba histolytica. Before the patient received his first injection of emetine, we obtained a sample of freshly passed mucus and brought it still warm to the laboratory, where it was thoroughly emulsified in warm physiological saline.

After 10 minutes in the thermostat a drop was examined and found to contain large numbers of amoebae. Some were already dead, but many were active, a fair proportion containing ingested red corpuscles. The following are the details of the experiment made to test the action of emetine on this emulsion.

6.00 p.m.   Put up samples with 1 in 1,000, 1 in 10,000 and 1 in 100,000 emetine.

6.15 p.m.   The amoebae in emetine 1 in 1,000 are practically all dead. Many are swollen up, with clearly visible nuclei. None are crawling, but a few are exhibiting ineffective protrusion of pseudopodia. One seen to extrude a large, clear pseudopodium and burst.

6.30 p.m.   The amoebae in emetine 1 in 100,000 and 1 in 10,000 are indistinguishable from those in the control emulsion. A few are dead in each case, and most are crawling actively.

6.55 p.m.   In 1 in 1,000 emetine nearly all the amoebae have disintegrated; the few still discoverable are dead.

7.00 p.m.   The samples with 1 in 100,000 and 1 in 10,000 emetine still show numerous actively crawling amoebae, with a proportion of dead ones; they are not perceptibly different from the control emulsion.

These results in no way support the suggestion that the action of emetine in vitro on Entamoeba histolytica obtained from the human bowel is more lethal or rapid than its action on our strains in kittens. Compare, for example, the experiment on human amoebae just quoted with the first experiments with emetine on strain A, recorded above under the date May 25. It is true that in some of the later experiments with strain A, and in the one satisfactory experiment with strain B, we observed a yet higher resistance to emetine; but we feel justified in attributing this to our greater care, with increased experience, in selecting healthy amoebae for such tests, rather than to change in resistance produced by passage through kittens. For a human strain we necessarily employed a dysenteric stool, and it is not surprising that the resistance to emetine of amoebae so obtained should resemble that of the weaker, rather than

that of the more resistant samples scraped directly from the mucous membrane of the kitten's intestine.

We have thus tested three possible explanations of the discrepancy between our results and those of Rogers, which led him to attribute to emetine a specific lethal action on Entamoeba histolytica far above that observed by us. All these explanations have been found wanting. We were not dealing with amoebae whose resistance had been artificially raised by previous emetine treatment; for the patient furnishing one of our strains was cured by further treatment with the same drug. Amoebae treated with higher concentrations of emetine than those used by Rogers not only retained their normal activity and appearance for prolonged periods, but also their power of infecting kittens. And, thirdly, the relatively high tolerance of emetine by our amoebae was not due to the fact that they infected kittens instead of men; for amoebae from an untreated human case survived for an hour a concentration of emetine described by Rogers as immediately lethal. One last possibility may be mentioned, only to be dismissed. It may be suggested that the emetine used by us was not a satisfactory preparation. We used several samples, obtained at different times; we detected no difference in their activity; and all were obtained from the same source as that used by Rogers. We are forced to the conclusion that Rogers' observation was fallacious, and can only suppose that the amoebae in his specimen had already become so enfeebled that they succumbed to emetine in dilutions which are harmless to those with unimpaired vitality.

The action of some other alkaloids and of various other substances

Though our experiments failed to demonstrate, for emetine and other alkaloids of ipecacuanha, an activity on amoebae in vitro at all comparable with that claimed by Rogers, they were obviously not without action, except in the case of psychotrine. It remained to be determined whether such action as emetine showed under these conditions was sufficient to account for its superiority, compared with other alkaloids, as

a remedy for amoebic dysentery in man. The failure of our attempts to cure dysentery in kittens with emetine, as described in the next section, led us to try the therapeutic effect of various other substances, after first examining the action of several of these on the amoebae in vitro. It seems worth while to place the results on record.

Quinine. When Lösch (1) implicated an amoeba (called by him "Amoeba coli," but without doubt the organism now called Entamoeba histolytica) as the causal agent in dysentery, he tried treatment with quinine on account of its reputation as a poison for protozoa and also because he found that quinine sulphate (1 part in 5,000) killed the amoebae in vitro "within one minute." Lavage with solutions of quinine has often been used in treating amoebic dysentery, but now usually as an adjunct to treatment with emetine. We made only two experiments on the action of quinine alone on the amoebae in vitro, and unfortunately none in which a direct comparison was made of its effect with that of emetine on the same emulsion. Both these experiments, however, were made on amoebae of strain A, and tests of the action of emetine and cephaeline are on record for about the same period of its history. The quinine experiments were made on emulsions from kittens 121 and 125, which were infected from kittens 117 and 119 respectively. Experiments on the action of emetine (and cephaeline) on amoebae from these last two kittens are on record above under dates September 28 and October 3.

October 4. Emulsion from kitten 121, strain A. Test of the action of quinine sulphate in saline.

1 in 10,000. 15 minutes. Amoebae apparently normal and crawling well.

60 minutes. Still crawling well, and not perceptibly different from the control.

1 in 2,000. 15 minutes. Practically all rounded up and motionless; a few just show slight spasmodic movement.

60 minutes. All certainly dead, and most disintegrated.

October 6. Emulsion from kitten 125, strain A. Another test of quinine sulphate.

1 in 100,000. 20 minutes. Quite normal.
>   40 minutes. Quite normal; indistinguishable from control.
1 in 10,000. Many amoebae killed immediately.
>   60 minutes. A large proportion have disintegrated. Of those
>   still intact many are still crawling actively.
1 in 1,000. All killed immediately.

A comparison of these two experiments with those of September 28 and October 3 will show that there was no wide difference between the action of quinine and that of emetine. Such difference as is indicated is in favour of quinine as rather the more potent; but it is not possible to attach any great importance to such an indication, as the tests were made on different emulsions. There is certainly no warrant in these results for regarding the direct action of emetine on amoebae as greater than that of quinine.

Harmaline. This is one of the alkaloids of *Peganum harmala.* It was shown by Raab (19) to have a lethal action on ciliated protozoa, which was intensified by exposure to light, like that of other fluorescent substances. This action was confirmed by Gunn (20), who showed further that the general pharmacological properties of harmaline resemble those of quinine. We experimented with a specimen of pure harmaline hydrochloride (obtained from Burroughs, Wellcome and Company). In one experiment a comparison of the action with that of emetine in the same emulsion was carried out.

May 28. Emulsion from kitten 31, strain A. Comparative test of emetine hydrochloride and harmaline hydrochloride. (The results with emetine are already given above under the same date. They are here repeated to facilitate comparison.)
1 in 100,000. Emetine. 25 minutes. Amoebae all living and normal. 3 hours. A few dead; the remainder as healthy as the control.
>   Harmaline. 7 minutes. Living, but sluggish; many show abnormally visible nuclei.
>   30 minutes. Many dead, a few still active.
> $2\frac{1}{2}$ hours. Most dead, a few living and apparently normal.

1 in 10,000. Emetine. 25 minutes. All living and normal.
           1 hour. All living and normal.
           3 hours. A fair number dead, but the rest normal and active.
     Harmaline. 7 minutes. All dead.

The effect of harmaline 1 in 250,000 was subsequently examined, with the result that a fair proportion of the amoebae were apparently killed even at this dilution; but as the sample was not put up until the amoebae had already been an hour in saline after removal from the intestine, the result is not comparable with the others. These show quite definitely that, under the experimental conditions, harmaline is a far more powerful amoebicide than emetine. Even 1 in 10,000 harmaline is more certainly lethal than 1 in 1,000 emetine, and 1 in 100,000 harmaline is not greatly inferior to the latter.

May 31. Emulsion from kitten 35, strain A. Test of harmaline hydrochloride.
1 in 500,000. More amoebae dead than in the control at the end of
             an hour; a definite, but weak effect.
1 in 100,000. 20 minutes. Many dead, a fair number still normal.
            40 minutes. Most dead, but a few still normally active.
            80 minutes. Still a few normal survivors.
1 in 10,000. 10 minutes. Practically all dead, a few just moving.
            80 minutes. Nearly all broken up; of the few remaining
            some still show weak, spasmodic movement.

There was no direct comparison with the effect of emetine on this emulsion, but in so far as comparison with the action of emetine on other samples is justifiable, the result supports the conclusion that harmaline has a much higher toxicity than emetine for amoebae under these conditions.

Cusparine hydrochloride. Cusparine is the principal alkaloid of Angostura bark, which has had some local reputation in therapeutics of a rather vague nature. A few years ago one of us (H. H. D.) observed, in the course of some experiments as yet unpublished, that cusparine had a considerably stronger lethal action on Paramecium than either quinine or harmaline. It is a weak base, and the solutions of its salts readily undergo hydrolytic dissociation on dilution with water, the free base

appearing as a milky colloidal suspension. We tested it on amoebae from the kittens in 1 in 100,000, 1 in 10,000, and 1 in 2,000 solutions, but could not detect an action of any kind. The amoebae survived as long in the 1 in 2,000 cusparine solution as in saline. The contrast between the indifference of amoebae to this substance, and the rapid death of Paramecia in highly dilute solutions of it, is not without interest.

Sodium desoxycholate. This pure bile-salt has recently been used by Mair (21) for the recognition of certain pneumococcal types, on which it has a specific solvent effect. The same observer found that it has the property of dissolving alkaloids such as quinine and optochin, which can thus be brought into alkaline aqueous solution. Such a solution has a more potent lethal action on pneumococci than its equivalent either of the bile salt or of the alkaloid alone. We were led to examine the action of sodium desoxycholate by a consideration of the distribution of amoebae in the infected bowel. As a rule the infection is sharply limited to the large intestine. It occurred to us that this might have some connexion with the presence of bile in the small intestine, and we were thus led to test the action of a bile salt on the amoebae in vitro. We took the opportunity of observing whether any reinforcement of the effect of quinine was produced by dissolving it with the aid of this salt, using a solution kindly supplied by Dr. Mair. The solution contained sodium desoxycholate and quinine in the proportions 10: 1.

Using first a pure desoxycholate solution, we found that, in a 1 in 1,000 solution all amoebae were immediately immobilized, and in a few minutes dissolved, so that no trace of them was discoverable in the solution. Obviously such an action could not be intensified by adding 1 in 10,000 quinine. In 1 in 10,000 desoxycholate a fair proportion of the amoebae became rounded up, but others were still active and looked normal after half an hour. The addition of 1 in 100,000 quinine, which by itself had no action on the amoebae (see above under quinine, experiment of October 6), made no perceptible difference in the action of 1 in 10,000 sodium desoxycholate; so that there was no evi-

dence of any mutual reinforcement. An attempt to use the desoxycholate therapeutically will be mentioned later.

Neosalvarsan. A number of favourable reports have been recently published on the action of salvarsan, and especially of neosalvarsan, in amoebic dysentery, though some observers have failed to detect any benefit from its use. To examine its effect on the amoebae in vitro, we chose neosalvarsan on account of its neutral solution. When added to the saline emulsion of amoebae in the proportion of 1 in 1,000 we could detect no effect of any kind, the amoebae retaining their vitality and activity quite as well as in the control. It is unfortunate that the opportunity was not taken of examining the infectivity of amoebae so treated. Hata (22), Castelli (23) and Gonder (24) have demonstrated that spirochaetes and trypanosomes, treated in vitro with salvarsan or neosalvarsan, though retaining their motility have lost their power of infecting a susceptible animal.

Tartar emetic. The recent demonstration of the curative effect of injections of tartar emetic in certain protozoal infections suggested the possibility of its having value in amoebic dysentery. Its action on amoebae in vitro was accordingly tested. Only one complete experiment was made, after a preliminary trial to ascertain the range within which the tests should be made.

June 12. Emulsion from kitten 42, strain A. Test of potassium antimonyl tartrate.

1 in 100,000. 15 minutes. Normal.
              30 minutes. A few amoebae dead, but the remainder indistinguishable from the control.
1 in 10,000. 15 minutes. A fair number dead, but others active.
              30 minutes. Some still crawling, but the majority dead.
1 in 1,000. 5 minutes. Many still crawling, but others motionless.
              15 minutes. Nearly all motionless, only a few making feeble ineffective movements.
              30 minutes. Nearly all broken up, the few still visible being dead.

So far as the effect in vitro is concerned tartar emetic has, therefore, an amoebicidal activity of a higher order than that

of emetine, as exhibited in most of the experiments with the latter.

Acriflavine (*Trypaflavin*). This dye (diamino-methylacridinium chloride), prepared by Benda (25) was shown by Ehrlich to have a powerful therapeutic action on trypanosome infections in mice. Browning '(26) has recently demonstrated its antibacterial properties, which, with its relatively low toxicity for mammalian tissues, give promise of its value as an antiseptic. A few preliminary experiments showed that it immobilized amoebae rapidly in relatively high dilutions. The following are records of more complete experiments.

June 29. Emulsion from kitten 51, strain A. Test of acriflavine.
1 in 10,000. Rapidly immobilizes all the amoebae, which stain yellow. None found moving after about 10 minutes.
1 in 100,000. Nearly all amoebae yellow and motionless in 30 minutes, but in each of 3 successive preparations one amoeba was discovered showing feeble movement, but no progression.
1 in 1,000,000. Again most of the amoebae became rounded up and motionless, but after 1 hour a few were found still crawling, and later, up to 3 hours, the active remainder persisted.
1 in 10,000,000. No difference from the control detected, in which the amoebae remained quite active up to 3 hours after preparation of the emulsion.
July 1. Emulsion from kitten 53, strain A. Test of acriflavine.
1 in 10,000. Less rapid in effect than in the previous experiment. Many amoebae retained fair motility after their contents had stained yellow. At the end of 1 hour all were motionless.
1 in 100,000. Most of the amoebae were immobilized in 1 hour. but a few remained quite active.
1 in 1,000,000. This dilution was put up an hour after preparation of the original emulsion, and stopped the movements of the amoebae with comparative rapidity, only a few remaining motile after 30 minutes.

It is evident that different samples of the amoebae show variations of sensitiveness to this dye similar to those observed

when dealing with alkaloids. In the experiment of July 1st it will be seen that the sensitiveness could be artificially increased by keeping the amoebae for some time in saline before the dye was added; after keeping in saline emulsion for an hour the amoebae, though apparently quite normal and highly active, are more rapidly affected by acriflavine 1 in 1,000,000 than the amoebae in the freshly prepared emulsion by a concentration 10 times as great.

It will be noted further that the above records speak of the amoebae as immobilized and stained yellow. Closer inspection convinced us that the yellow staining was confined to the endoplasmic granules. The motionless amoebae did not show the same tendency to break up as those killed with alkaloids, nor were the nuclei visible with abnormal distinctness. The question arose whether they were indeed killed, or merely paralysed. It was put to the test of experiment with the emulsion from kitten 53. The experiment recorded above (July 1) showed that all the amoebae of this emulsion were immobilized by 1 in 10,000 acriflavine. Of the remainder of this emulsion 4 cc. were kept as a control, and to another 4.5 cc., an hour after the emulsion had been prepared, was added 0.5 cc. of acriflavine 1 in 1,000 making a dilution of 1 in 10,000 in the mixture. After a further incubation of an hour no active amoebae could be found in this; all were motionless and stained yellow. In the control emulsion, which had now been 2 hours in the thermostat, the amoebae were still highly active. The 4 cc. of control emulsion were injected per rectum into a new kitten no. 61, and the 5 cc. of emulsion containing 1 in 10,000 acriflavine into a similar kitten, no. 62. Kitten 61 began to pass blood, mucus and amoebae on July 4, and died on July 7; kitten 62 passed blood, mucus and amoebae on July 5 and died on July 11. It might be suggested that the treatment with acriflavine had slightly delayed the onset and retarded the course of the infection; but even this is doubtful, for both the incubation period of 4 days and the 6 days between onset and fatal issue are well within the limits of variation with untreated samples of this strain. The only safe conclusion is that acriflavine in the strength used paralyses the

amoebae, but makes little difference in their subsequent power of infection.

Blood-serum. There were several reasons for examining the action of blood-serum on the amoebae. We thought that possibly serum would prove a better medium for preserving the amoebae in vitro, and testing the action of drugs on them, than a watery saline solution. In any case it would be desirable to test the action of the various alkaloids, etc., when dissolved in serum, as the blood and lymph must generally be their medium of contact with the amoebae when they are used therapeutically. A few trials soon showed us, however, that it is impossible to keep the amoebae alive and active in serum, which appears to kill them in vitro with a quite surprising rapidity.

The serum which we first tried was obtained from a full-grown cat, and it seemed possible that the action observed was related to the comparative resistance of such adult animals to amoebic infection. We therefore collected the blood from an etherized kitten, separated the serum from the clot and placed in cold storage. Two days later it was used in the following experiment.

June 26. Kitten 49, which was in the terminal stage of its infection with strain A, was killed. The large intestine showed an almost uniform, superficial ulceration of the mucous membrane, which was covered with a layer of mucus containing very large numbers of good, active amoebae. A very uniformly ulcerated segment was cut out, and divided into two portions. From one of these the amoebae were scraped into 5 cc. of saline, from the other into 5 cc. of the kitten's serum mentioned above, and both tubes were placed in the thermostat at 36°. In the saline all the amoebae remained extremely active for several hours; and even after 6 hours many were still active and intact. In the serum most of the amoebae lost their motility in 10 to 15 minutes; after 30 minutes a large proportion had already disintegrated and the remainder were mostly motionless; after 60 minutes only a few could be found which were still active. These, however, did not appear to be further affected for some time, but all were dead at the end of 6 hours. The action of the serum of the kitten was not perceptibly less than that of serum from the grown cat.

There could be no question, in this case, of a paralysis of the amoebae, for many were disintegrated. It seemed desirable, however, to test the infectivity of the minority which apparently survived.

June 22. Kitten 47, in the terminal stage of infection with strain A, was killed. The scrapings from the mucous membrane of its large intestine were partly emulsified in saline as usual, and injected into new kittens 49 and 50. The remainder was emulsified in fresh serum from a normal dog and placed in the thermostat. A specimen examined immediately showed abundance of healthy, active amoebae. After 30 minutes, however, another sample showed nearly all the amoebae dead, and many disintegrated. After 50 minutes no active amoebae could be found. 10 cc. of the serum-emulsion were then injected in the usual way into kitten 51.

Kittens 49 and 50 began to pass blood and mucus containing amoebae on June 24. Kitten 49 was killed on June 26, before the appearance of terminal symptoms, and used for the experiment above; kitten 50 was showing symptoms of approaching death on June 28, and was killed accordingly. We may therefore take the "control" incubation period and interval between onset and death as 2 and 4 days respectively.

Kitten 51 began to pass blood, mucus and amoebae on June 25, and became moribund on June 30, when it was killed. A typical ulceration of the large intestine was found and the amoebae from it were used for testing the action of trypaflavin in the experiment recorded above under date June 30. The incubation-period and interval between onset and death are in this instance 4 and 4 days. So that treatment with serum, which caused disintegration of most of the amoebae, and inhibited the activity of the remainder, had only the effect of prolonging the incubation period; and even this was within the limits normal for the strain.

Mercury succinimide. Mercury being, like other heavy metals, to a considerable extent excreted through the mucous membrane of the large intestine, might be expected to have some effect on an amoebic infection. One of us (C.D., 31) recently examined the faeces of a patient infected with Entamoeba histolytica, who was undergoing a course of mercurial treatment for syphilis, but no effect on the amoebic infection could be detected. At the time when we were making these experiments, however, it seemed desirable to test the action of a mercury compound, and we chose the succinimide, as its solutions do

not coagulate albuminous fluids. The results illustrate the sharpness with which a lethal concentration can be determined for a stubstance which kills the amoebae rapidly. A striking feature of its action was the fixation of the amoebae by the higher concentrations with such rapidity that at first sight they looked normal, with pseudopodia extended as in the act of crawling. Only when further inspection showed that all remained quite motionless, and that the nuclei were fixed, was a correct interpretation of these appearances arrived at.

June 2. Emulsion from kitten 34, strain A. Test of mercury succinimide.

1 in 1,000 and 1 in 10,000. Appear to kill almost instantaneously, so that samples examined as soon as possible after mixing show the amoebae motionless and fixed, with extended pseudopodia.

1 in 100,000. Kills more slowly and only partially. After 20 minutes the majority are rounded up and motionless, but some are still quite active, and remain so up to 1 hour, when observation ceased.

June 7. Emulsion from kitten 39. Test of mercury succinimide. Dilutions of 1 in 1,000 and 1 in 10,000 were again immediately lethal, the amoebae dying with pseudopodia extended.

1 in 100,000. Again killed only a proportion of the amoebae, which became spherical before dying, the rest remaining active. Intermediate concentrations were therefore tested.

1 in 20,000. Killed all immediately, like 1 in 10,000.

1 in 30,000. Most were killed, but not all immediately, and a small proportion resisted this concentration, and remained active.

1 in 40,000 and 1 in 50,000. Resistant survivors were found in increasing proportion.

It seems probable, therefore, that the absolutely lethal concentration of this substance lies between 1 in 30,000 and 1 in 20,000.

Copper alanine. The crystalline copper salts of aminoacids, such as glycine and alanine, have recently been mentioned by Shaw-Mackenzie (27) as of possible therapeutic interest, on account of their action on protozoa and their relatively low toxicity for mammals. Dr. Shaw-Mackenzie asked us to test

such compounds on Entamoeba histolytica, and kindly gave us samples. Copper alanine was tested on two occasions.

September 12. Emulsion from kitten 99, strain B. Test of copper alanine.
1 in 100,000. 15 minutes. Normal.
      1 hour. Many amoebae dead, the remainder moving feebly.
1 in 10,000. 15 minutes. Normal.
      1 hour. All dead.
1 in 1,000. 15 minutes. All alive but rather sluggish.
      . 1 hour. All dead.
    Control. 1 hour. All alive and highly active.
October 7. Emulsion from kitten 133, strain A. Test of copper alanine.
1 in 100,000. 20 minutes. The majority crawling actively, but more dead than in control.
      1 hour. Numbers smaller than in control, a proportion having evidently disintegrated; survivors as active as control.
1 in 10,000. 20 minutes. The majority dead, a few showing slow amoeboid movements.
      1 hour. Many disintegrated, the remainder nearly all dead, but a few just moving feebly.
1 in 1,000. 20 minutes. Nearly all dead and disintegrating; a very few moving feebly.
      1 hour. All dead, and nearly all disintegrated.

Chaparro amargosa. An extract of the bark and twigs of this plant (Castela nicholsoni), the "bitter bush" of the Mexicans, has recently been found effective in the treatment of cases of amoebic dysentery which had proved refractory to emetine. Through the kindness of Dr. P. I. Nixon, of San Antonio, Texas, who has recently published several papers (28) on its therapeutic use, we obtained a consignment of the drug. Nixon found that the detannated fluid extract killed Entamoeba histolytica (obtained from human liver-abscess pus) in extremely high dilutions (up to 1 part in 10 million) in a few minutes. We prepared a decoction from the powdered drug in accordance with the indications given in Nixon's papers, which state that it should be made of such strength as to have the "colour of moderately weak tea."

Our decoction was made by boiling 1 part of powdered drug in 10 of water. The tannin was removed by precipitation with lead acetate, and the small excess of lead precipitated by passing sulphuretted hydrogen. The solution was boiled to remove sulphuretted hydrogen, filtered from lead sulphide, and then concentrated to one-half the original volume.[5] This clear, detannated extract had the intense, bitter taste of the original decoction, which apparently owes its action to a neutral bitter principle. It is of interest to note that Simaruba, the bark of which has long enjoyed a very similar reputation in the treatment of dysentery in the Old World, is closely related botanically to the "chaparro amargosa," and also contains a neutral bitter principle.

For the test on amoebae the "detannated" extract was neutralized, and 0.9 per cent of sodium chloride added. On July 23 kitten 71, infected with strain A, was killed, and an emulsion made of the amoebae scraped from its large intestine. The emulsion contained a good number of healthy active amoebae and was unusually free from epithelial cells and other contamination. After smaller proportions had failed to show any effect it was mixed in equal parts with the chaparro extract, and the mixture placed in the thermostat. Even after an hour the amoebae in this mixture were thoroughly active, and showed no difference from those in the control emulsion. There is thus the most complete discrepancy between our observation and those of Nixon. We are unable to estimate the relation between the strength of our extract and that of the detannated fluid extract which he employed; but, while it is possible that our extract was less concentrated, and that the procedure used by us for removing tannic acid might have weakened the activity, the contrast between lethal effect in a concentration of 1 part in 1 million and no effect at all in a concentration of 1 part in 2 cannot be explained along such lines. If we suppose that the extract used by Nixon contained 1 per cent of the active principle—a very generous assumption—it would be active in his experiments in a dilution of 1 part in 100 mil-

[5] We are indebted to Dr. A. J. Ewins, who kindly made this purified extract for us.

lions.   In our own experiments with a large variety of substances we find no ground for believing that any substance whatever could affect the vitality of Entamoeba histolytica in dilutions of this order, within the time limits set by the survival of the organism in vitro.   Nor can we suppose that our extract of the "Chaparro," however reduced by treatment, would contain less than 1 part in 50 millions of an active principle having any significance for the action of the drug.   It might be suggested that we had by chance obtained an inactive sample; but this supposition is excluded by the fact that several patients, carrying an infection with Entamoeba histolytica resistant to emetine, have, at our suggestion, been treated with an extract from the same sample of drug, made similarly to ours but not detannated, and that these patients have thereby been cured.   We see no escape from the conclusion that Nixon's observation on amoebae in vitro was in some way fallacious.   The therapeutic effects of Chaparro, according to our own results, must either be due to its action on the patient rather than on the amoebae, or to some constituent which is removed by precipitants of tannic acid.[6]

V. ATTEMPTS TO TREAT AMOEBIC INFECTION IN THE CAT

The experiments described in the preceding section led us to the conclusion that the direct effect of emetine, on otherwise uninjured specimens of Entamoeba histolytica, is very much less than previous observations seemed to indicate; and that other alkaloids, with no such reputation for specific action on amoebic infection, have a lethal effect on the isolated amoebae as great as, or greater than, that of emetine.   It was obviously desirable to compare the therapeutic effects of the other ipecacuanha alkaloids which appeared more powerfully amoebicidal than emetine, and which, as will be shown in a later paper, are all considerably less toxic than emetine for the mammal.   We naturally expected to be able to use as a standard of therapeutic action the effect of emetine on the animals infected with our two

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[6] Nixon, however, expressly states (1915, p. 577) that the detannated fluid extract "is no less potent than the original fluid extract."

strains of amoebae. This expectation was disappointed. Various methods of administration were tried; emetine hydrochloride was given by hypodermic injection, started as soon as the evidence of infection appeared; the double iodide of emetine and bismuth was administered by the mouth in daily doses of 4 to 10 milligrams; both hypodermic injections of the hydrochloride and oral administration of the double iodide were begun a few days before the infecting injection was given, and continued daily after it; and finally the large intestine was filled daily with 10 cc. of a 1 in 10,000 solution of the hydrochloride. Yet in no instance could we detect any prophylactic or curative effects. The failure was, perhaps, particularly noteworthy in the case of strain B, since the patient from whom it was derived had meanwhile been cured of his infection by a course of emetine bismuthous iodide. Two experiments may be quoted as examples.

(1) *May 4.* Kittens 16 and 17 each took 4 mgm. of emetine bismuthous iodide by mouth.

May 5. Kittens 16 and 17 and two control kittens, 18 and 19, were each given per anum 5 cc. of an emulsion of amoebae of strain A obtained from kitten 15, which was killed in the terminal stage of infection. Kittens 16 and 17 were again given 4 mgm. each of emetine bismuthous iodide by mouth.

May 6. Kittens 16 and 18 are passing blood-stained mucus and amoebae; kittens 17 and 19 normal.

May 7. Kittens 16 and 18 are still passing mucus and amoebae and kitten 17 has begun to do so; kitten 19 still normal. Kittens 16 and 17 each receive 10 mgm. of the double iodide by mouth.

May 8. Kittens 16, 17, 18 all passing abundant amoebae; kitten 19 has passed a trace of blood-stained mucus. Kittens 16 and 17 again take 10 mgm. of the double iodide.

May 9. Kitten 18 dead. The usual ulceration of the large intestine found post-mortem. Kitten 16 and 17 still passing abundant amoebae. Kitten 19 normal.

May 10. Kitten 16 dead. The usual ulceration found post-mortem. Kitten 19 normal. Kitten 17 still passing abundant amoebae.

May 11. Kitten 17 still passing amoebae. Kitten 19 normal.

May 15. Kitten 17 very ill. Killed. Extensive ulceration of the

lower half of the large intestine was found, and scrapings from the ulcers showed immense numbers of healthy, active amoebae. Kitten 19 still normal.

Against the fact that one of the controls, no. 18, dies on the 4th day after infection, while the kittens treated with emetine before and after infection died on the 5th and 7th days respectively, must be put the fact that the second control, no. 19, failed to take the infection. Clearly no protective or curative action could be attributed to the treatment,

(2) *May 18.* Kitten 19, unsuccessfully injected in the above experiment, and two new kittens, kittens 27 and 28, each received an injection of 5 cc. of emulsion of amoebae of strain A, obtained from the intestine of kitten 22, which had just been killed. All gave evidence of successful infection on May 19. On May 20, kitten 19 was given 5 mgm. of emetine hydrochloride hypodermically. This caused vomiting, and on the 21st and 22nd it was given injections of 3 mgm. only. Kittens 27 and 28 died on May 22, kitten 19 on May 23, having continued throughout to pass abundant mucus with numerous active amoebae. All these kittens showed typical amoebic ulceration of the large intestine on post-mortem examination, and there was no indication that the treatment of kitten 19 had in any way altered the course of the infection. Yet this kitten had received injections of emetine which, in proportion to its body weight of 500 grams, would correspond to injections of about 10 grains, 6 grains and again 6 grains for a man of 60 kgm. Lest the dosage should have failed through excess, other kittens were treated with 1 mgm. doses, with no more influence on the infection.

With kittens infected with strain B we observed a similar complete absence of curative effect from administering emetine hypodermically as the hydrochloride, or by the mouth as the double iodide with bismuth. It will be remembered that the patient from whom this strain was obtained was cured of his infection by treatment with the double iodide. The course of the infection which it produced in kittens being, on the whole, slower than that produced by strain A, there was plenty of time for an effect of the drug to become apparent before the general condition of the animal became seriously affected. The absence of result is thus the more significant.

Similar attempts were made to treat infected kittens with methyl-psychotrine, demethoxy-emetine and N. methyl-eme-tine; the infection in all cases being strain A. Since all these alkaloids are much less toxic for the mammal than emetine, and more active on the amoebae in vitro, it was hoped that a thera-peutic effect would be obtainable with one or more of them. The most that could be detected, however, was an apparent slight delay of the fatal issue; and the variation shown by the course of infection in different untreated animals, even when they are simultaneously infected with equal amounts of the same emulsion, makes it impossible to attach any significance to such a result. The following are typical results:

Kittens 53 and 54 were infected on June 28, and both passed amoebae on June 30. Kitten 53, untreated, died on July 1. Kitten 54 was given 10 mgm. daily of N. methyl-emetine sulphate hypodermically on June 30, July 1 and July 2. It died on July 3, having continued to pass blood, mucus and active amoebae throughout. The typical ulceration was found post-mortem.

Kittens 121 and 122 were similarly infected on September 28. Both passed amoebae on September 30, and kitten 122, which was not treated, continued to do so till it died on October 3. Kitten 121 passed nothing on October 1, but again mucus and amoebae on October 2, when 10 mgm of methyl-psychotrine sulphate were injected hypodermically. The injection was repeated on October 3, but the dysentery continued unchecked, and the kitten died on October 4. The intestine showed the usual ulceration and contained abundant active amoebae.

Kittens 147 and 148 were similarly infected on November 15. Both began to pass blood, mucus and amoebae on November 16. Kitten 147, which was not treated, died on November 18. Kitten 148 was given 50 mgm. daily of demethoxyemetine sulphate on November 16, 17 and 18 by hypodermic injection, and died on November 19, having continued to pass abundant mucus and amoebae throughout.

In each case the treated kitten dies a day or two later than the control, but the difference is, in any case, trivial, and quite possibly accidental.

A similar absence of any definite modification of the course of the infection was observed in kittens treated with harmaline,

injected hypodermically or into the large intestine per anum; and with neosalvarsan (60 mgm.), tartar emetic (2.5 mgm.), and copper alanine (10 mgm.) injected intravenously, the kittens being etherized during the intravenous injections. Danysz's new preparation called "Luargol"—a salvarsan compound containing antimony and silver in addition to arsenic—was also given intravenously to an infected kitten, in a dose of 10 mgm. in alkaline solution. The course of the infection was again not modified. Sodium desoxycholate having proved so rapidly lethal to the amoebae in vitro in a concentration of 1 in 1,000, the effect of washing out the large intestine of an infected kitten twice daily with this bile-salt in a solution of 1 in 500 was tried. A rather more definite retardation of the course of the infection appeared to result from this treatment; but a parallel experiment, in which the bowel of another injected kitten was washed out twice daily with physiological saline, showed as great a delay of the fatal termination, in comparison with the result in the untreated control animal.

Amoebic dysentery in cats, therefore, as produced by the strains of Entamoeba histolytica which we succeeded in establishing, is evidently an extremely intractable infection. Even heroic doses of emetine, far exceeding in relation to the weight of the animal those which produce a rapid subsidence of the symptoms in man, failed to influence perceptibly the course of the infection. It is impossible, therefore, to attach any significance for human therapeutics to similarly negative results with other substances. A positively beneficial effect would have been strongly suggestive, but such we have failed to obtain. We think it unlikely that other animals will prove more suitable for such therapeutic investigation, when artificially infected with human strains of Entamoeba histolytica. It appears to us that the greatest hope of advance in the therapeutics of amoebic dysentery by animal experiment lies in utilizing the natural infections which have been described as occurring in dogs and monkeys, and producing a dysentery of a less acutely fatal type than that which we have described in our kittens. Meanwhile, since this line of investigation was not

open to us, it seemed desirable to have clinical trials, on human
cases of infection, carried out with some of the substances which,
having a lower toxicity for mammals than emetine and a greater
direct action on amoebae, gave promise of therapeutic value.
At present only two of these substances have been tried clinical-
ly.   Major J. C. Meakins, C. A. M. C., kindly undertook the
trial of methyl-psychotrine at the Hampstead Military Hospital,
and Prof. J. W. W. Stephens that of N. methyl-emetine at Liv-
erpool.   We are greatly indebted to these gentlemen for under-
taking the trials, and for their permission to publish the follow-
ing notes of their results.

   1.   *Methyl-psychotrine.*   Two chronic carriers of Entamoeba histolyt-
ica were treated with this drug per os, receiving gradually increasing
doses until as much as 9 grains daily was being given.   One case re-
ceived 54 grains in total amount, the other 108 grains.   Both were
examined almost daily during treatment, and were found to be passing
cysts or amoebae regularly throughout.   Neither suffered from any
sickness or diarrhoea.   The drug appears, therefore, to have been
without action on either the patients or their amoebae.   Both these
cases were subsequently treated with emetine bismuthous iodide (3
grains daily for 12 consecutive days), which promptly, and apparently
permanently, rid them of their infections.   (The examinations were
made with great care by Miss E. M. Jepps.)
   2.   *N. Methyl-emetine.*   Professor Stephens informs us that treat-
ment with N. methyl-emetine was given to several chronic carriers of
Entamoeba histolytica whose infection had proved refractory to emetine
in various forms, and was similarly not eradicated by methyl-emetine.
Of two carriers not previously treated with emetine, one was apparently
cured by a course of methyl-emetine, while the other relapsed after a
similar course (1 grain daily for 15 days).   An attempt to give 3 grains
daily was abandoned on account of the vomiting which it caused.

VI. DISCUSSION OF RESULTS

   We believe that our work represents the first systematic attempt
to use kittens artificially infected with Entamoeba histolytica
from man for the purpose of therapeutic experiment.   From that
point of view it has failed; and one of our objects in placing

it on record is to warn others, who may have a similar investigation in view, of the probability of a like failure. But apart from this failure, the results seem to have some interest as an indication of the necessity for revising the current simple conception of the action of emetine in the cure of amoebic dysentery.

As early as 1891 Warden (29), speaking of the action of ipecacuanha in dysentery, was able to say: "that the active remedial agent in the drug is the emetine is a question not open to controversy." And by the same year Tull Walsh (30) had also convinced himself, by careful clinical experiment, of the correctness of this view. At that date, however, the aetiology of dysentery was still a matter of dispute, and the chemistry of the ipecacuanha alkaloids in a very elementary stage. It is of interest to note that even a very recent device for administering emetine was anticipated in principle by Warden, who advocated the administration of the crude "emetine" of those days (mixed emetine and cephaeline) as the insoluble double iodide with mercury, in which form it could be swallowed by the patient without being vomited. Both Walsh and Dymock (vide Warden, *loc. cit*) used this compound with success. These important observations apparently received little recognition, so that the action of ipecacuanha in dysentery was generally regarded as debatable, and treatment with preparations of the drug freed from its emetic alkaloids was still widely practised until about 1912. By that date the bacteriology and protozoology of dysentery and the chemical differentiation of the ipecacuanha alkaloids were much further advanced. Vedder (12),[7] who was able to use practically pure preparations of emetine and cephaeline, found that both had a powerful amoebicidal action, which the extracts of ipecacuanha, freed from alkaloids, did not possess, although the activity of a total extract from the untreated drug was apparently not strictly proportional to its alkaloidal content. A little later Lyons (34) also studied the action of ipecacuanha extracts on free-living amoebae from cultures, and came to a

[7] A preliminary account of these experiments was first published in 1911 (Bull. Manila Med. Soc.)

different conclusion. According to Lyons "the experiments
with ipecac on amoebae *in vitro* fail, thus far, to explain its
clinical value in the treatment of amoebic dysentery." But
Rogers, shortly afterwards, obtained results very similar to
those of Vedder, using as his test object samples of living Ent-
amoeba histolytica obtained direct from human cases of acute
dysentery. As a result of these observations Rogers then tried
the effect of emetine hydrochloride, administered hypodermically,
in amoebic dysentery; and his results, with those of many sub-
sequent observers, have established beyond all question the
claim of the alkaloids to be the therapeutic agents in the ipeca-
cuanha treatment. The ensuing improvement in the treatment
of amoebic dysentery has been so conspicuous, that the experi-
mental observations which led up to it have obtained an author-
ity, which would possibly not have been accorded to them if they
had been critically considered apart from their clinical conse-
quence. In the light of the latter there has hitherto been no
reason to question their soundness; and the action of ipecacuanha
in curing amoebic dysentery seemed to occupy, in the completeness
and simplicity of its explanation, an almost unique position among
chemotherapeutic problems. If emetine hydrochloride in a
dilution of 1 in 100,000 would kill Entamoeba histolytica in a
few minutes, there was no reason to look further than this
direct action for an explanation of the disappearance of amoebic
dysentery when 30 to 60 mgm. of the salt were injected daily
into the patient.

The supposition that emetine acts thus directly on amoebae
is implicit in the preliminary account, recently published by
Pyman and Wenyon (18), of experiments made by another
method on the antiamoebic action of ipecacuanha alkaloids.[8]
They tested the power of emetine, cephaeline and various
derivatives, some of which we have also examined, to inhibit
the growth of a free living amoeba, when added in varying con-
centrations to the nutrient agar-medium. They found that
emetine, cephaeline, and their N. methyl derivatives prevented

[8] The authors kindly allowed us to see the paper before its publication

the growth of the amoebae when added to the medium in proportions of 1 in 1000 or 1 in 10,000, and had some restrictive effect on multiplication in a concentration of 1 in 100,000.   The action of psychotrine under the same conditions was hardly perceptible.

When our own results are considered, it appears to us impossible to maintain this simple conception of the action of emetine. In sufficient concentration emetine exhibited, indeed, a power of killing Entamoeba histolytica suspended in saline solution, but the concentration required to kill the amoebae, within the period of their survival in vitro, was in all cases far beyond the highest concentration which could be produced in the circulation of a patient without killing him.   It appears to us, further, that an explanation of the therapeutic effect of emetine, on the basis of such action as it shows on isolated amoebae, would only be permissible if it were shown that other alkaloids, which have not the specific effect on amoebic dysentery, have a less powerful effect on amoebae in vitro.   So far from this being the case, we find that quinine which is not a specific for dysentery, has a somewhat more powerful action on amoebae than emetine; we find further, what is even more significant, that so closely related a substance as methyl-psychotrine, differing from emetine only by two hydrogen atoms, is somewhat more strongly amoebicidal in vitro, but apparently quite devoid of any therapeutic action in human infection, although its low toxicity for man allows it to be given in relatively enormous doses.

This contrast in therapeutic value between emetine and methyl-psychotrine stands in the way of many of the possibilities of theoretically reconciling the weak action of emetine on isolated amoebae with its curative action in dysentery. It might be thought, for example, that the amoebicidal action is comparatively slow, and that the limit set to observation, by the period of survival of the amoebae in saline solution, does not allow the effect of high dilutions to be demonstrated; or that the relatively weak direct lethal action is an index of a restrictive action on multiplication in very much weaker concentrations.   Again, it might be suggested that emetine in

high dilutions, though it does not kill the amoebae, so weakens them that they are unable to resist the natural defensive measures of the host. All these considerations, however, should apply with equal force to the closely related, and rather more actively amoebicidal methyl-psychotrine.

There is clearly some other factor, in the cure of dysentery by emetine, than the alkaloid and the amoebae; and that other factor must be supplied by the host. The participation of the host in the process is demonstrated by our observation that emetine has no appreciable effect on the course of amoebic dysentery in the cat, while it cures it in man, even when the same strain of amoebae is present in the two hosts. (See our experience, recorded above, with strain B.)

There are several ways in which the host may be supposed to take part in the action.

1. We may take refuge in the idea, which has so often been invoked to explain similar discrepancies between action in vitro and curative effect, that emetine does not act as such, but that something is formed from it in the body which is much more powerfully amoebicidal. It would be possible to make a plausible case for this idea, by pointing to the fact that emetine and cephaeline, which cure dysentery, are completely saturated substances, while psychotrine and methyl-psychotrine contain an unsaturated linkage, and by suggesting that the body breaks them down along different lines. We may state frankly our view that this idea is not worth discussion, unless it can be stated in more precise chemical terms. The oxidative break-down of these alkaloids in the laboratory leads to the same products (Pyman). Moreover the conception is difficult to reconcile with the fact that emetine apparently acts more powerfully on amoebic infection of the colon, when it is liberated in the intestine, than when it reaches the site of action through the circulation.

There is evidence already that a similar or even greater discrepancy, between action on the amoebae in vitro and curative effect in dysentery, will occur in the case of other substances. We have recorded above our complete failure to detect any action

on amoebae with the extract of "Chaparro amargosa." At the same time we had placed a quantity of the same batch of the drug in the hands of hospital authorities, who were able to substantiate Nixon's account of its value in eradicating infection with Entamoeba histolytica, from cases which had proved refractory to thorough treatment with emetine. It will hardly be possible to postulate the formation in the body of an amoebicidal derivative from every substance which cures dysentery but has a relatively weak action on the isolated amoebae.

2. We may frankly abandon the idea that the curative powers of emetine are due to action, more or less directly, on the parasite, and suppose that it acts primarily upon the host. This view has not the attractive simplicity of that which attributes the effect to direct amoebicidal action, but it is in harmony with facts which the other conception fails to explain.

a. We may refer again to the puzzling contrast between emetine and methyl-psychotrine. The only difference which we detected between their amoebicidal actions was that methyl-psychotrine was slightly the more active. When we come to test their action on mammals,[9] however, we find that emetine is very much more toxic, and, in particular, that the pronounced emetic action, the irritating effect on the alimentary mucous membrane, is practically wanting in methyl-psychotrine. We find then that of the alkaloids, emetine, cephaeline and methyl-psychotrine, those which are curative in dysentery, emetine and cephaeline, are distinguished from the non-curative methyl-psychotrine, by greater toxicity for the host, not by greater toxicity for the amoebae.

It may be noted that N. methyl-emetine, which was approximately equal to methyl-psychotrine in amoebicidal action, has been found to have some curative effect in dysentery, though, so far as results are available, less than that of emetine. At the same time it was found to have a distinct emetic action, though less than that of emetine.

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[9] A detailed account of the pharmacology of this series of alkaloids will be published later by one of us.

We hope to have other alkaloids of the series tested for their effect on dysentery. Especially interesting will be the action of demethoxy-emetine—the most actively amoebicidal of the group and, at the same time, the least toxic to mammals. We hazard a prediction that it will be found to have no curative action.

b. Emetine completely failed to cure, or even to retard the course of amoebic infection in the cat, even when the infecting strain was one which a thorough course of emetine eradicated from the human donor. (See our experience with strain B.) Since the parasite and the drug were identical, the difference in the results of the treatment in the two cases must be attributed to a difference between the hosts in their response to the drug.

c. The apparently specific action of emetine in suppressing infection with Entamoeba histolytica, while failing to rid the host of Entamoeba coli and Entamoeba nana (31, 32, 33), may seem at first sight to be in favour of a direct and highly specific action on the parasite. To us it seems rather to be a further point in favour of action on the host. We have not found it practicable to study the effect on the other species of entamoeba in vitro; but the fact that other observers, working with the much less closely related free-living amoebae, have found that emetine has on these an effect of the same order as that which we observed on Entamoeba histolytica, makes it highly improbable that its direct effect on Entamoeba coli and Entamoeba nana would be conspicuously less.

When we remember that Entamoeba histolytica is an obligate tissue-parasite, which can live and multiply only by invading the host's tissues, while Entamoeba coli and Entamoeba nana live freely in the contents of the bowel, we see again that an action on the host, preventing further invasion of tissue, is the effect which would most easily account for the conspicuous difference between the effects of emetine on the different kinds of infection.

When we pass to a consideration of the manner of action of emetine on the host, which can explain its beneficial effect on amoebic dysentery in man, we have practically no facts on which to build a theory. We must suppose that the resistance

of the host's cells to the cytolytic action of the amoebae is in some way increased, the natural defensive reaction to the invasion in some way promoted. It may be that the action of emetine in this direction is facilitated by its concentration in the alimentary mucosa, to which some features of its pharmacological action seem to point. Such points, however, are better left for discussion in the light of further knowledge. The conclusions which, in our opinion, can fairly be deduced from our observations, are (1) that emetine does not cure amoebic dysentery by virtue of an intense direct amoebicidal action, but that the host must in some way coöperate in the effect; and (2) that there are indications pointing to a connexion between that property of emetine and cephaeline which gives them an emetic action, and their therapeutic efficiency in amoebic dysentery. These seem to us points of some importance for the future development of the therapeutics of amoebic infection. It would seem natural, in the absence of such indications, to proceed along the lines made familiar by Ehrlich in other connexions, and to endeavour to discover derivatives or homologues of emetine having stronger "parasitotropic" properties, as shown by lethal action on amoebae, and weaker "organotropic" properties, as shown by diminution of general toxicity or of tendency to cause vomiting and gastro-intestinal irritation. We believe that investigation along such lines will probably lead to disappointment. We have quoted one case (methyl-psychotrine) in which it has already done so. A fuller knowledge, a more complete analysis, of the pharmacological action of emetine and related alkaloids on the mammalian organism may possibly lead to a clearer conception of the manner in which the host contributes to their therapeutic action on amoebic dysentery.

VII. SUMMARY

1. Two strains of Entamoeba histolytica were established in kittens; one, from vegetative amoebae injected per anum, was transmitted through 43 passages; the other, from cysts injected into the stomach, through 6 passages. In neither

case did the strains alter perceptibly in character with successive passages.

2. Various alkaloids and other substances, including the natural alkaloids of ipecacuanha and artificial derivatives therefrom, were tested on the amoebae in vitro. Emetine and the other alkaloids of ipecacuanha exhibited no characteristically high toxicity for the amoebae, as compared with that of some other alkaloids. Certain samples of Entamoeba histolytica from our kittens survived the action of 1 in 1,000 and even 1 in 100 emetine, for periods up to 1 hour. Amoebae which survived treatment by 1 in 1,000 emetine were found to be still capable of infecting kittens.

3. Experimental dysentery in kittens was refractory to all kinds of treatment. Neither the ipecacuanha alkaloids, nor other substances having a powerful action on the amoebae in vitro, could cure the infection or definitely modify its course.

4. Methyl-psychotrine, a natural alkaloid from ipecacuanha which is more toxic for Entamoeba histolytica, when tested in vitro, and much less toxic for mammals than emetine, has been tested clinically on cases of amoebic infection in man. It appeared to be entirely devoid of therapeutic action, though given in relatively very large doses.

5. On the basis of these results it is suggested that the theory of the mode of action of emetine in amoebic dysentery needs reconsideration. Alternatives are discussed to the theory of direct amoebicidal action, which seems to be no longer tenable; and it is suggested that the therapeutic efficacy of emetine is a result of its action upon the host rather than upon the parasite.

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THE SALICYLATES IX. THE QUESTION OF SALICYLURIC ACID IN SALICYL URINES

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CONTENTS

I. INTRODUCTION AND CRITICAL REVIEW OF PREVIOUS WORK

The possibility of salicyluric acid playing an important rôle in the elucidation of the mechanism of action of salicylates in rheumatic fever led to the study of this excretory product. It is said to occur in urine after the administration of salicylates to the extent of about 80 per cent of excreted salicyl in man; but is said to be absent in the urine of dogs. The chemical formula is usually given as $C_6H_4.OH.CO-NH.CH_2COOH$, by which it resembles hippuric acid.

The presence of salicyluric acid in urine was first reported by Bertagnini (1) in 1856, who obtained a crystalline residue by ether extraction which when exposed to a current of air at 140° to 150°C. yielded two products. That which volatilized was declared to be salicylic acid and the remaining non-volatile portion, salicyluric acid, because when purified it possessed the following properties; melting point 160°, it was readily soluble in ethyl-acetate and alcohol but less so in ether; more soluble in hot water, but almost insoluble in cold water; the taste

was bitter, and possessed an acid reaction. The elemental composition including nitrogen answered the theoretical for salicyluric acid. In a report by Gnehm (2) to the Swiss Chemical Society in 1875, it is stated that Piccard was able to confirm the results of Bertagnini, although he objects to the method of purification (by means of sublimation) recommended by Bertagnini as insufficient to give pure salicyluric acid. No data are presented, but according to Neubauer and Vogel (3), who quote Piccard, the separation of the two acids, i.e., salicylic and salicyluric, is accomplished by crystallization from ether and benzene, the salicylic acid crystallizing first. According to Stockman (4) salicyluric acid is insoluble in boiling benzene, and according to Baldoni, in chloroform. These solvents were used by these investigators to separate salicylic from salicyluric acid in urinary residues obtained by ethereal extraction. However, in neither case were the products sufficiently studied so as to identify them as salicyluric acid. U. Mosso (5) requires the use of ethyl acetate besides ether for the complete extraction of both the salicyl acids and claims to be able to separate salicyluric from salicylic acid in his quantitative method for the estimation of salicylate in urine, but Mosso also did not identify the supposed product as salicyluric acid. On the other hand, Baas (6) claims that salicyluric acid can be completely extracted from acidified salicyl urine by ordinary ether alone.

Using the method of Mosso, no salicyluric acid could be detected by Wiley (7) in the urines of subjects taking salicylate.

Nencki (8) claims to have recovered salicyluric acid from urine by ether extraction of the acidified extractive obtained from urine treated with neutral lead acetate and then with alcohol. The melting point of his product was 159°C., and the elementary analysis for carbon, hydrogen and nitrogen correspond to the theoretic, but it is not clear how the figure for nitrogen was obtained. Nencki states that the decomposition of salicyluric acid in urine into glycocoll easily occurs. Another method mentioned by Nencki consists of direct extraction from urine without previous treatment with lead acetate and alcohol,

and then the two acids are separated by means of their different solubilities in ether. Apparently the lead acetate treatment is not so essential. Using a method similar to that of Nencki, Lesnik (9) obtained a product which melted at 160°, and from the analysis of the carbon and hydrogen content he calculated the nitrogen content (without actual determination) and these figures put together corresponded to the theoretic formula for salicyluric acid. Lesnik doubted if salicyluric acid is the only salicyl constituent in urine.

According to Hildebrandt (10) and Neuberg (11), salicylic acid is for the most part excreted unchanged, a part being excreted as ethereal sulphate, salicyluric acid, salicyl glycuronic acid and oxysalicylic acid. Gnehm also speaks of the passage of much unchanged salicylic acid into urine, and this he regards as being of therapeutic interest. Apparently the ratio of the two acids found by Gnehm was about 1 gram of salicylic acid to 0.5 gram of salicyluric acid in a liter of urine.

The work of Baldoni requires somewhat more detailed consideration, for it represents the latest effort to demonstrate the existence of salicyluric acid in urine. In his earlier work, Baldoni (12) asserts that he could demonstrate salicyluric acid in the urine of man but not in dog's urine. In dog's urine, two other salicyl compounds were obtained, one with nitrogen, i.e., uraminsalicylic acid, $C_{15}H_{16}NO_8$, the other without nitrogen, ursalicylic acid, $C_{15}H_{14}O_8$. In uraminsalicylic acid Baldoni claims that the nitrogen is not in the form of amino acid because he could obtain no test for the amino group, but the tests are not mentioned. Both compounds occur in urine together.

Both salicyl compounds isolated by Baldoni are said to give an azure color with ferric chloride. This was the chief differential point from salicylic acid which gave a violaceous color. Baldoni admits that his ethereal extract residues were not entirely free from impurity. Could not this have modified the typical violet to azure color? I believe I have occasionally observed a similar modification of the iron salicyl color in the presence of impurity, but attached no significance to it. Baldoni found that alcoholic extracts of cartilage, muscle and lung

also gave an azure color with iron, from which he concluded that these tissues contained the two new salicyl compounds.

In human urine, a small quantity of the new compounds is present, but apparently there were no definite observations on this. It is interesting to note also that Baldoni claims that salicylic and salicyluric acid are precipitated when an excess of chloroform is used to shake out the urine. Baldoni claims that dosage has some influence on the appearance of these compounds in urine. When the dosage is not too large in the dog, the greater part of salicyl appears in the urine as such, and only a small part as the two new salicyl compounds. Baldoni states that salicyluric acid has never been verified for the dog.

In more recent work, Baldoni (13) has withdrawn a former claim made by him regarding the isolation of salicyl glycuronic acid from the urine of individuals receiving salicylate. For this he now substitutes the two new salicyl compounds, i.e., the uraminsalicylic and ursalicylic acids. Baldoni regards Neuberg's new product to be the same as his ursalicylic acid. He also attaches no importance to the use of lead acetate in the isolation of salicyl compounds from urine.

In a still later publication, and regarding human urine, Baldoni (14) claims that the data on the excretion of salicyl and salicyluric acids as reported by Mosso and others are discordant. Using his own method, Baldoni claims that salicyl elimination is mostly completed in twenty-four hours after administration. After the administration of sodium salicylate (about 5 gram), the proportion of the excreted acids is:

$$\frac{\text{salicyluric}}{\text{salicylic}} = \frac{53.28 \text{ per cent}}{25.11 \text{ per cent}} = \frac{2.12}{1} \text{ in twenty-four hours}$$

No identification of salicyluric acid is reported; the separation of the two acids was assumed to be complete by means of solvents. Baldoni, however, concludes that the greater part of administered salicyl is eliminated as salicyluric acid, that individual variations exist and whether the conjugation can be facilitated or interfered with remains unsolved.

According to some investigators, it is also interesting to note that the meta and para-hydroxybenzoic acids, although differing chemically from the ortho acid (salicylic acid) only with respect to the position of the (OH) group, do not give rise to salicyluric acid in the organism. Baumann and Herter (15) claim to have isolated a nitrogen-containing compound in urine after the administration of paraoxybenzoic acid to dogs. This is not definitely regarded as a glycocoll conjugation product, and no quantitative analyses for nitrogen are given. Baumann and Herter concluded that the three oxybenzoic acids, namely, the oxy- (salicylic acid), para-, and meta- form in part ethereal sulphates and in part compounds analogous to hippuric acid, a part of them being excreted unchanged, and only a small part of the paraoxybenzoic acid is decomposed into phenol with the formation of phenolsulphuric acid.

It is generally accepted that only the ortho-oxybenzoic acid (salicylic acid) is therapeutically efficient in rheumatic fever.

Concerning the synthesis of salicyluric acid, Bondi (16) claims to have successfully accomplished this from hydrazine hydrate, methyl salicylate, nitrous acid, glycocoll and alkali. The melting point of this product was between 170° and 172°C.; another product gave 230° to 231°C. However, E. Fischer (17) objects to Bondi's products on the basis of impurity and he synthesized salicyluric acid from carbomethoxy-salicylic acid hydrochloride and glycocoll, but did not study its properties, i.e., melting point, solubility, etc. Complete elementary analysis of only one of the products is reported, and this agreed with the theoretical for salicyluric acid. These syntheses were made under special conditions, and, even if correct, do not necessarily indicate that salicyluric acid occurs in the animal organism. Bondi's method will be described later in the paper together with my attempt at the synthesis of salicyluric acid and the results obtained.

From all of this, it is apparent that our knowledge concerning salicyluric acid is far from satisfactory. I have attempted to isolate salicyluric acid from urine of individuals receiving sodium salicylate, but without success. Several different human individuals, some of which received full therapeutic doses of

sodium salicylate and others only moderate doses, have been studied. The urines of a number of animals (dogs and a cat) also were studied. The methods of Bertagnini, U. Mosso, Baldoni, Stockman, and Nencki, and various modifications of these, and also other methods, were applied to urines under various conditions, i.e., freshly voided, preserved, standing and decomposed, with and without the use of heat. These will be presently considered in detail and the data presented.

II. IDENTIFICATION PROCEDURES

It is obvious that before a product may be accepted as salicyluric acid it must possess certain physical and chemical properties that are distinctive and different from salicylic acid with which it is closely associated. Essentially this resolves itself into a determination of the melting point, estimation of the nitrogen content, and, if nitrogen is present, it must be shown that it exists in the form of a-amino acid nitrogen. In addition is is desirable to know the solubility in different solvents, since it is upon this that the separation of salicylic and salicyluric acid is based by many investigators. However, it was not possible to study this with all the products because of the limited yields. It is very essential that the products be pure, for so far as nitrogen is concerned, this might be derived from the amino nitrogen of hippuric acid occurring normally in urine, or other nitrogenous constituents. The lack of information on this and the nitrogen content of the alleged salicyluric products in previous investigations is notorious, and I deem it necessary, therefore, to set forth in detail a description of the procedures used by me, even though they may appear elementary. These were as follows:

Determination of the melting point. The arrangement of Menge (18) was used, using liquid petrolatum (Stanolind), instead of sulphuric acid. The same thermometer was used (without correction) throughout and was checked against a certified instrument, and gave the melting point of 156°C. with a pure recrystallized specimen of Kahlbaum's salicylic acid.

Solubility. Approximately equal quantities of the product were added to equal quantities of the different solvents, and this was expressed as readily soluble, slowly soluble or insoluble. There were no essential differences in solubility of the products tested by me, and, therefore, this phase of the investigation merited no further consideration except merely to determine the general behavior of each product whenever the yield permitted.

Nitrogen content. This is one of the most essential steps in the identification of salicyluric acid. Salicyluric acid according to the formula generally assigned to it, should contain 7.18 per cent of nitrogen. This was determined by the usual Kjeldahl procedure, using $\frac{N}{100}$ sulphuric acid and $\frac{N}{100}$ alkali with methyl orange in a few and methyl red in most experiments as indicators. When no mention is made of the method used, it is understood to be the Kjeldahl method. In a few instances, however, the Folin colorimetric procedure was used. The small quantities of the products to which I was restricted by the limited yields were frequently controlled by the analysis of similar quantities of hippuric acid and occasionally synthetic salicyluric acid in the same way. The quantity of the product used for nitrogen estimation was usually in the neighborhood of 0.05 gram, and the actual range with all products was from 0.0124 to 0.101 gram.

Detection of a-amino nitrogen. Tests for this became essential when the presence of nitrogen was demonstrated, in order to ascertain whether or not the nitrogen represented impurity or was derived from the amino radicle which salicyluric acid should contain as one of the components of the conjugation product glycocoll. Qualitatively, this was tested for by the triketone hydrate (ninhydrin) reagent of Abderhalden, using 0.2 to 0.5 cc. of a 1 per cent solution, and boiling for one minute in the usual way. The sensitivity, 0.005 to 0.05 mgm. in 1 cc. (19), of the test was great enough for my purpose.

The crystalline salicyl residues (usually about 0.05 gram) were usually hydrolyzed in a boiling water bath and over a direct flame with 5 to 10 per cent hydrochloric acid, in some cases concentrated phosphoric acid, for adequate periods (three to twelve

hours) of time, using a similar quantity of hippuric acid for control and in the same way. The hydrolyzed mixtures were then neutralized to litmus and the ninhydrin test was applied. In a number of instances the hydrolyzed product became yellow, and in order to exclude this as a possible interference with the development of the ninhydrin test further controls were made by adding a small quantity (about 0.02 gram) of synthetic salicyluric acid to such mixtures. In that event, the tests were invariably positive (brilliant blue color) when otherwise they were negative (yellow to reddish color), and, therefore, showed definitely that the yellow impurity was not responsible for negative ninhydrin tests.

Purification. This was attempted by treatment with animal and blood charcoal (Merck's), recrystallization from water and other solvents, washing with cold water by centrifugalization, reprecipitation and other procedures, according to the state of purity of the individual product. This varied considerably, but as a rule the products were highly colored (brown), particularly those portions designated as "salicyluric acid" in the methods that were studied.

Iron-salicyl test. The presence of the salicyl group was tested for by the application of a 2 per cent ferric alum solution (previously boiled and filtered) to the crystals on a watch glass, or in some cases to solutions of the crystals. Ordinary salicylic acid gives the well known typical violet color; synthetic salicyluric acid also a violet and phenol a blue color. Whenever the distillation-colorimetric method for the quantitative estimation of salicyl is mentioned, this refers to the method previously described by Thoburn and Hanzlik in the Journal of Biological Chemistry, 1915, **23**, 163.

Dosage of salicyl. This is expressed in the protocols as salicylic acid, which was administered in all cases in the form of sodium salicylate.

III. THE DESCRIPTION AND APPLICATION OF VARIOUS METHODS
PROPOSED FOR THE ISOLATION OF SALICYLURIC ACID,
AND RESULTS

The following methods have been carried out as closely as possible in conformity with the description of the authors. In some cases there were modifications and these will be indicated in the protocols.

I. Method of Bertagnini (1)

The urine is evaporated to a small volume, the fluid portion being separated from any solid matter by filtration. This is then acidified with hydrochloric acid and repeatedly extracted with ether. The ethereal solutions are allowed to evaporate and the remaining crystals are purified by recrystallization from boiling water and treatment with animal charcoal. The salicylic acid is separated by volatilization brought about by exposing the entire mass to a current of air at 140° to 150°C. The non-volatile portion consists of salicyluric acid and is further purified by treatment with boiling water and animal charcoal.

Experiment 12. Urines of subjects M. D. and P. J. H., each taking 2.6 grams of salicylic acid were collected until salicyl free; mixed and divided into two portions, one portion (A) of which was treated according to Bertagnini's description, and portion (B) in the same way, except that the urine was not evaporated in the preliminary step., and ethyl acetate was used in addition to the ether to extract the salicyl.

No salicyluric acid was obtained from portion A, for the product entirely volatilized at 110° in air.

After special purification by treatment with animal charcoal and recrystallization the product from portion B, two sets of crystals were obtained. The long thick needles had a melting point of 156–158°, the shorter thin needles gave a melting point of 164° and were partly soluble in chlorofrom and benzene. Both sets of crystals gave positive iron-salicyl tests, and negative tests with ninhydrin, while hippuric acid under the same conditions gave a positive test for a-amino nitrogen. A mixture of both crystals was washed with hot benzene and chloroform for the removal of salicylic acid. A small yellowish residue

remained, and on further purification gave a melting point of 156°;
positive iron-salicyl test, and completely volatilized on the water-bath.

It is seen that the salicyl residues obtained from urine by
Bertagnini's method behaved like ordinary salicylic acid as to
volatility, melting point, when the product was sufficiently pure,
and with two of the products the tests for a-amino-nitrogen
were negative.

2. Nencki's method (2)

The freshly voided urine is treated with small quantities of
neutral lead acetate so that no precipitate of lead sulphide ap-
pears in the filtrate when treated with hydrogen sulphide.   The
filtrate is carefully evaporated on the water-bath and the remain-
ing syrup extracted with absolute alcohol.   The alcoholic fil-
trate is then evaporated and the remaining residue is acidified
and extracted with ether.   After evaporation of the ether a
yellowish acid syrup remains, which on the following day gives
rise to a mass of crystals composed of fine needles.   The crystals
are decolorized by boiling with animal charcoal and on repeated
crystallization from ether are obtained snow white.   The melt-
ing point of the purified product was observed by Nencki to be
159°C.   This method was considered better than the following,
which Nencki states to have repeated with saligenin.

The concentrated (by evaporation) urine is acidified and extracted
with ether without the addition of lead acetate.   In this way both the
salicylic and salicyluric acids are obtained.   In order to separate the
two acids, use is made of their different solubility in ether.   I have not
tried out this procedure because similar steps are involved in other
methods.
The urines from two animals, dogs 20 (experiment 26) and 22 (experi-
ment 28) receiving 1.08 and 1.8 gram salicylic acid, respectively, and
from one human individual, patient 37 (experiment 27) receiving, 4.5
grams salicylic acid, were treated according to Nencki's method.   When
the residues from alcohol extraction were acidified with 10 per cent
hydrochloric acid, voluminous white precipitates, flocculent and some-
what feathery, were formed in all the urines.   These did not consist
cf salicyl because they were insoluble in ether, ethyl acetate and 98

per cent alcohol, and did not give positive salicyl tests with iron.   They were soluble in sodium hydroxide and precipitable with hydrochloric acid. Some of the precipitate from the urines of dogs 20 and 22 turned whiter on ignition, did not volatilize, and, therefore, was neither salicylic nor salicyluric acid.   The lead precipitate also contained no salicyl.

One of the products from the urine of dog 20 was a thick reddish brown smear and in spite of repeated treatment with animal charcoal did not crystallize for four months.   The material gave a bluish-wine color with ninhydrin, that is, semi-positive for a-amino nitrogen, and resembling a color obtainable with any urinary residue.

After further purification by treatment with animal charcoal and recrystallization, the product from the patient's urine consisted of long thin transparent crystals with melting point at 153–155°; nitrogen content was 0.87%; iron-salicyl test positive.   The products from the mixed urines of dogs 22 and 20 consisted of long needles with a yellowish tinge; iron-salicyl test positive.   These crystals melted at 153° and gave a nitrogen content of 1.24 per cent.   All of the products from both the animal and human urines gave negative tests with ninhydrin, and when synthetic salicyluric acid was added to the products and treated in the same way positive tests were obtained, showing that the urinary residues contained no detectable a-amino nitrogen.   The solubility of each of the crystalline residues was about the same, namely, they were readily soluble in alcohol, ether, ethyl acetate and hot water, slowly soluble in chloroform and benzene.

It is permissible to conclude that the urinary salicyl products obtained by Nencki's method were not salicyluric acid.

3. Method of U. Mosso (5)

First the mucoid and other substances are removed from the urine by precipitation with neutral lead acetate.   The precipitate is washed until salicyl-free.   Then the filtrate is treated with an excess of ammonia and lead acetate and heated.   The precipitate which now contains the salicylic and salicyluric products is filtered off and decomposed with ammonium carbonate or sulphuric acid, filtered again, and the precipitates washed until salicyl-free.   From the filtrate the salicyl acids are now removed by repeated extraction with small quantities of a mixture of ether and ethyl acetate.   This requires about six to eight

extractions. The ethers are allowed to evaporate spontaneously from a suitable dish and the crystalline residue is weighed. The whole is then heated on a water bath until the weight becomes constant; it is weighed again and the weight of the second residue corresponds to salicyluric acid. The difference between the weights of the two residues corresponds to salicylic acid which had volatilized.

Both human and animal urines were treated according to Mosso's method. Experiment 7 will be described separately because of the special attempts at purification.

Experiment 7. Twenty litres of an old urine (preserved with toluene) obtained from several different individuals receiving sodium salicylate was evaporated on the water-bath and treated according to Mosso's method. About 7 grams of a brown crystalline material was obtained. This was purified in the usual way by treatment with animal charcoal and hot water and the filtrate allowed to recrystallize. The clean white crystals gave a melting point of 155.5°, completed at 157°; the ninhydrin test was positive. The crystals were now further purified by dissolving in hot water and a little sodium hydroxide and the pre-cipitate with hydrochloric acid. The crystals were now repeatedly washed with cold water by centrifugalization and dried in a current of air. Glistening snow-white crystals were obtained. The melting point began at 155°, completed at 156°. 0.025 gram of the crystals yielded 3.4 per cent of nitrogen = to about 47 per cent of theory for salicyluric acid; ninhydrin test was negative, while hippuric acid under the same conditions gave a positive test.

The remainder of the crystals was now placed on the water-bath as directed by Mosso for the removal of the salicylic acid, the non-volatile residue representing "salicyluric acid." However, the crystals com-pletely volatilized, leaving the evaporating dish clean.

Experiments 10, 25, 29, and 31. These experiments with Mosso's method may be grouped together, since in these the method was carried out in the same way. The quantities of sodium salicylate received by subjects B and C were not recorded. The doses of salicylic acid in the form of sodium salicylate administered to the following were: patient 37, 4.5 grams; dog 20, 0.85 gram; cat 21, 0.72 gram.

Weighed portions of the crystalline residues left after the evapora-tion of the ethers of the ethereal extracts were evaporated on the water-bath for the removal of salicylic acid and the portions remaining behind

corresponding to "salicyluric acid" were controlled by colorimetric estimation according to the distillation—colorimetric procedure. All of the nonvolatilized residues were brown, non-crystalline smears. For convenience all of the results are presented in the following table:

TABLE

NUMBER OF EXPERIMENT	URINE OF	QUANTITY OF CRYSTALLINE RESIDUE TAKEN	SALICYLURIC ACID		SALICYLIC ACID BY DIFFERENCE	REMARKS
			By weight after evaporation	By colorimetric estimation of the non-volatile residue*		
		gram	gram	gram	gram	
10	Subject B	0.0401	0.0107 = 25%	0.000141† = 0.35%	0.0294 = 73.3%	
	Subject C	0.0301	0.0153 = 50.8%		0.0148 = 48.9%	Duplicates of same urine
	Subject C	0.0885	0.0418 = 47.2%		0.0467 = 53%	
	Subject C	0.0317	0.0259 = 81.7%	0.00606‡ = 19%	0.0101 = 31.9%	Duplicates of same urine
	Subject C	0.0963	0.0478 = 49.6%	Trace; too small to estimate	0.0485 = 50.4%	
25	Dog 20	0.1554	0.0588§ = 37.8%	0.002 = 1.3%	0.0966 = 62.1%	Color with ferric alum not characteristic of salicyl; phenol-like
29	Patient 37	0.8236	0.2612§ = 31.7%	0.004 = 0.48%	0.5624 = 68.3%	
31	Cat 21	0.9542	0.6058§ = 63.5%	0.100 = 10.5%	0.3484 = 36.5%	Color with ferric alum not characteristic of salicyl; phenol-like

* The material was first hydrolyzed with concentrated phosphoric acid and then distilled, and the distillate estimated colorimetrically with ferric alum.

† Expressed as salicyluric acid = to 0.0001 gram salicylic acid.

‡ Expressed as salicyluric acid = to 0.0043 gram salicylic acid.

§ A small quantity of each of the "salicyluric acid" residues was hydrolyzed with 1 cc. of 10 per cent hydrochloric acid for four hours; neutralized and 0.25 cc. of 1 per cent ninhydrin was added and boiled in the usual way. The material of experiment 25 gave a yellowish (negative test), from experiments 29 and 31 a brown color with ninhydrin (negative), while a small quantity of synthetic salicyluric acid under the same conditions gave a blue (positive test), showing that the urinary salicyl residues contained no demonstrable a-amino nitrogen, and, therefore, cannot be regarded as a salicyluric acid.

It is seen that both animal and human urines behaved alike, and that the non-volatile portions corresponding to "salicyluric acid" contained variable though very small and nearly negligible quantities of salicylic acid when controlled by the colorimetric estimation. No a-amino-nitrogen was demonstrable by the ninhydrin test in three of the experiments and, therefore, the conjugation product, glycocoll, is excluded. The physical state of these sticky brown residues undoubtedly influences the rate and extent of the volatilization of the salicylic acid, as Baldoni has suggested. Accordingly, the method is not suitable for quantitative estimation of salicyl.

4. Stockman's method (4)

The urine is evaporated almost to dryness, thoroughly mixed with hydrochloric acid to set free the salicylic and salicyluric acids; mixed with fine sand and percolated with ether and ethyl acetate until the acids are extracted. On evaporating the ether the residue is a dark yellow syrup. This is dissolved in boiling water, heated with animal charcoal, filtered hot and evaporated to dryness. The salicylic acid is dissolved by boiling benzene, in which it is very soluble, leaving behind the salicyluric acid. The salicylic acid so obtained should be nearly colorless and is crystalline. The salicyluric acid may be obtained also practically pure by dissolving in boiling water and crystallizing out. Stockman states that salicyluric acid so obtained is in fine crystals, has a slightly bitterish taste with no trace of sweetness, is almost insoluble in benzene and is much less soluble in ether than salicylic acid. Its solubilities in water, alcohol and chloroform do not differ much from those of salicylic acid.

Seven experiments were performed, and of these six (experiments 1, 2, 3, 4, 13 and 16) were on human urines (from patients 6, 10, C, 7 and subjects J and S) and one (experiment 24) on dog's urine (dog 20). All the urines except in experiment 16 and the dog urine were treated according to the directions in Stockman's method. The urine of patient 7 (experiment 16) was not evaporated, but was extracted directly after acidifying with ethyl acetate and ether, and, in general,

heat was avoided as much as possible throughout. The total dosage of salicylic acid for the patients ranged from 2.58 to 13.76 grams., and for the dog it was 0.94 gram. The salicylic acid and salicyluric acid products may be described as follows:

. *Salicylic acid or benzene soluble portion:* The melting point of the cleaner products from human urines ranged from 154–156° and when mixed with pure salicylic acid the melting point was 155°. A set of crystals associated with a set melting at 156° melted at 134°, and another set from the same dish did not melt at 255°. Two sets of impure crystals from the human urines melted at 115° and 145°, respectively, and upon further purification at 155°. One set of crystals melted at 117°, contained 9.42 per cent of nitrogen, but gave a negative ninhydrin test, while the addition of synthetic salicyluric acid to some of the product gave a positive test. The other products were not tested for the presence of nitrogen, nor with ninhydrin. All of the products gave positive iron-salicyl tests. The solubility of practically all of the products was about the same, namely, they were readily soluble in alcohol, ethyl acetate, hot water, chloroform and benzene, slowly soluble in cold water. These products may be regarded as salicylic acid of varying degrees of purity.

"Salicyluric Acid" portion. The products corresponding to salicyluric acid were difficult to purify in spite of repeated treatment with animal charcoal, hot water and recrystallization. Human urines: One set of gritty, sandy crystals melted at 145°, and on further purification left a residue which did not melt at 250°, and gave no color with iron. Another product that gave a positive iron-salicyl test did not melt at 250–365°. One urine yielded no crystals, but only a brownish oily smear. Another urine gave a set of crystals with melting point of 157–161°, and these were soluble in ethyl acetate, slowly in ether and benzene; ninhydrin test was negative, while the control with synthetic salicyluric acid added to some of the urinary salicyl was positive. The properties of still another product were, melting point 155°, charring at 183°; nitrogen content 9.09 per cent = to 126.6 per cent of the theory for salicyluric acid; the Soerensen formol titration with 0.005 gram gave 58 per cent of theory while a similar quantity of hippuric acid gave 93 per cent, but the ninhydrin test was negative and a control with hippuric acid was positive. The products were generally readily soluble in alcohol, ethyl acetate and hot water, slowly soluble in chloroform, benzene and cold water. Some of them possessed a bitterish sour taste.

Dog's urine: A fair crop of white crystals was obtained and these gave a melting point of 130°; nitrogen content of 45 per cent; ninhydrin test was negative; both crystals and a solution gave no test with ferric alum; were readily soluble in alcohol, ethyl acetate and hot water, nearly insoluble in benzene, ether and cold water; taste was pungent and sour. From the negative iron test these crystals did not contain salicyl.

The urinary salicyl products obtained by Stockman's method can not be regarded as salicyluric acid when judged by their melting points, nitrogen content and response to tests for a-amino-nitrogen.[1]

5. Method of Baldoni (21)

Baldoni criticises Mosso's method on the grounds that salicylic acid does not volatilize completely in the presence of impurities such as occur in urinary extractives, and he also regards Mosso's results as discordant. The latter objection is also raised by Baldoni against Stockman's results. Baldoni, therefore, proposes a different way of extracting the alleged salicyluric acid. This is based on a a difference of solubility of the two acids in chloroform and ordinary ether, salicylic acid being first removed by chloroform leaving behind the "salicyluric acid" to be removed with ether. In detail, the method is as follows:

The urine is evaporated on a water bath with the aid of a fan to a syrupy consistency. The syrupy fluid is then acidified with sulphuric acid (40 per cent) and extracted repeatedly (at least three times) with ether. The ether is allowed to evaporate

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[1] In a personal communication dated October 20, 1915, Professor Stockman informs me that he did not determine the melting points of his products, but identified them by their solubilities, by testing for nitrogen, i.e., for ammonia by soda-lime, and for their action on yeast and some common bacteria. It does not appear to me that such tests are sufficiently specific and definite. For instance, almost every one of my products (by any method, including Stockman's), when tested, showed the presence of nitrogen, but in no instance was it possible to demonstrate the presence of a-amino-nitrogen, which is necessary in order to establish that the product contains glycocoll. In other words, the nitrogen, when present, is apparently derived from some impurity.

and the residue is treated with hot water and heated with animal charcoal, filtered, the filter is washed and the filtrate is concentrated to a volume of about 50 cc.   It is also permissible to extract the dry residue left after evaporation of ether directly with chloroform.   After cooling the concentrated filtrate is extracted with about 120 cc. of chloroform, shaking vigorously in a separatory funnel.   If no precipitate is formed, the first chloroform layer is removed, and a further addition of 80 cc. of chloroform is made.   The residue left after evaporation of the chloroform contains all of the salicylic acid.   According to Baldoni, the precipitate which may be formed at this time, i.e., during extraction with chloroform, consists of salicyluric acid, and again it may remain in solution in the aqueous layer.

The aqueous layer remaining after treatment with chloroform is now repeatedly (at least three times) shaken with ether, and the residue left after the evaporation of the ether consists of salicyluric acid.   Apparently no further purification is necessary.

This method is said to have been controlled by the addition of salicyluric acid and salicylic acid to urine, and the separation of the two is reported successful.   However, Baldoni does not state in any of his publications how he obtained or prepared the salicyluric acid that was used in his controls, or whether he used the synthetic product.   There is no definite assurance, therefore, that Baldoni worked at all with salicyluric acid in his controls.   It is admitted by Baldoni that the method is inexact, but the advantages claimed by him are ease of execution and correspondence to clinical need.

The urines from two patients (C, experiment 13 and F, experiment 14) and two dogs (20, experiment 23, and 20 and 23, experiment 30) were treated according to Baldoni's method.   The dosage of salicylic acid was as follows; 5.2 grams for each of the patients and 0.85 gram for dog 20, experiment 23, and 1.08 grams for dog 20 and 0.9 gram for dog 23, experiment 30.

Chloroform soluble or salicylic acid portion.   Repeated purification by treatment with charcoal and recrystallization of a product from patient C finally gave short, needle-like, slightly brown crystals which melted at 155° to 157°.   Most of the crystals of the ethereal residue

were removed by treatment with chloroform so that very little was left corresponding to salicyluric acid. The crystals obtained from patient F were not studied.

The urines of both animals yielded crystals (after considerable purification) which melted at 155 to 157°. Both gave positive iron-salicyl tests. One product gave a nitrogen content of 0.177 per cent and the other, 1.06 per cent, but both products gave negative tests with ninhydrin and when synthetic salicyluric acid was hydrolyzed with these products the test was positive. Their solubilities were practically alike, that is, the products were readily soluble in alcohol, ether, ethyl acetate, chloroform, benzene and hot water, slowly in cold water.

"*Salicyluric Acid*" *portion.* Both the human and animal urines yielded products which were difficult to purify, but after repeated treatment with charcoal and recrystallization they were subjected to the tests. Human urines: One product had a melting point of 157 to 160°; nitrogen content of 164 per cent of theory; the ninhydrin test was negative. The other product had a melting point of 157 to 160°; taste was bitter and sour; the nitrogen content was 126 per cent of theory for salicyluric acid and the ninhydrin test negative. Controls with synthetic salicyluric acid and hippuric acid added to both products gave positive tests with ninhydrin. Both products gave positive iron-salicyl tests.

Dog urines: The crystals of one product were tinged with yellow and had a melting point of 155 to 159°; contained 0.272 per cent of nitrogen. The other product was white, crumbly and non-crystalline and had a melting point of 155° and contained 2.65 per cent of nitrogen. Both products gave negative tests with ninhydrin, while the addition of synthetic salicyluric acid to the products, and treated in the same way, gave positive tests. One product gave a blue color (azure of Baldoni?) and the other a typical salicyl violet with ferric alum. Their solubilities were the same, namely, they were readily soluble in alcohol, ether, ethyl acetate, hot water, somewhat less readily in benzene, chloroform and cold water.

It may be concluded that all of the salicyl residues corresponding to both salicylic acid and the salicyluric acid portions obtained with Baldoni's method behaved like an impure salicylic acid.

6. Miscellaneous procedures

Freshly voided urines. The urine of subject S (experiment 5) receiving 4.3 grams of salicyl, was extracted with chloroform only, and the purified crystals from this extract gave a melting point of 145°; nitrogen was absent by Lassaigne's test and the salicyl-iron test was positive. The urine of patient 9 (experiment 15) was acidified and extracted directly with ethyl acetate and ether. No attempt was made to separate salicylic from "salicyluric acid," but two sets of crystals could be discerned in the residue left after evaporation of the ethers. One set consisted of thick needles; the others were thin needles. Both had the same melting point, i.e., 154 to 156°. The nitrogen content of a mixture of the two sets of crystals was 0.104 per cent; titration by the Soerensen formol method gave 0.4 per cent of amino nitrogen, but the ninhydrin test was negative, while hippuric acid gave a positive test.

The urine of patient 12 (experiment 18), receiving 8.75 grams salicyl, was extracted by a combination of Nencki, Stockman and Baldoni methods, and the products in each case were specially purified by treatment with animal charcoal, recrystallization and washing with cold water by centrifugalization so as to free them as much as possible of a yellowish impurity. The benzene soluble portion (salicylic acid portion of Stockman) yielded short, white, needle-like crystals with melting point of 155.5°; nitrogen content of 0.004 per cent and the iron-salicyl test was positive. The chloroform soluble portion (salicylic acid portion of Baldoni) yielded scaly white crystals with melting point of 155° and nitrogen content of 0.52 per cent; iron-salicyl test, positive. The portion left behind, after treatment with chloroform and benzene, corresponded to "salicyluric acid," and consisted of white, partially crystalline scales with melting point of 155.5 to 157°; a mixture of these crystals and pure salicylic acid gave a melting point of 154 to 155°; the nitrogen content with the Folin colorimetric method was 0.004 per cent; ninhydrin test was negative; iron-salicyl test positive, and the crystals were readily soluble in alcohol, ether and ethyl acetate, almost insoluble in hot water, benzene and chloroform.

The urine of dog 26 (experiment 32), receiving 1.35 grams salicyl, was evaporated to a pillular consistency, acidified and then extracted directly with ethyl acetate and ether. The interesting feature about the residue left after evaporation of the ethers was a dark brown oil, which, on continued extraction with hot water, yielded several crops

of needle-like crystals until finally a nearly salicyl-free brown smear remained. The oily material was freely soluble in alkali and easily precipitated by hydrochloric acid. The crystals were purified by boiling with Fuller's Earth and blood charcoal, and recrystallization. They now consisted of clean, white, short needles with melting point of 153 to 155°; nitrogen content of 0.62 per cent; ninhydrin test was negative, but appeared positive when synthetic salicyluric acid was hydrolyzed with some of the same urinary salicyl residue; iron-salicyl test positive. The crystals were readily soluble in alcohol, ether, ethyl acetate, chloroform, benzene and hot water, slowly in cold water.

Old human urines. In experiment 8, a collection of old standing (for several months) urines, preserved with toluene, from several different patients was acidified and extracted directly with ethyl acetate and ether. The chloroform extract of the brown crystalline residue left after evaporation of the ethers yielded white crystals with melting point of 155 to 159°; Lassaigne's test for nitrogen was negative, and the iron-salicyl test was positive. The undissolved portion left after treatment with chloroform was treated with ethyl acetate, and the white crystals that separated from this extract were so small in amount that only the iron test was performed and this was positive for salicyl.

Another urine (experiment 19, patient 12), which had stood about a month, preserved with toluene, was extracted in the same way as the urine in experiment 8. The crystals were specially purified by treatment with animal charcoal, filtered and recrystallized. The product was dissolved in sodium hydroxide and reprecipitated with hydrochloric acid. This precipitate was redissolved in sodium hydroxide and the alkaline solution was now shaken several times with chloroform, petroleum ether, ether and ethyl acetate to remove the brown color, but this was unsuccessful, so the solution was acidified and extracted with ether until salicyl-free and recrystallized from water. White needle-like crystals were obtained and these gave a melting point of 155 to 156°; 0.044 per cent of nitrogen and the ninhydrin test was negative; iron-salicyl test was positive. The supernatant fluid from these crystals eventually yielded a crop of brownish crystals which resisted decolorization. They were recrystallized from alcohol and gave a melting point of 159°; nitrogen content of 0.26 per cent and the ninhydrin test was negative.

It is seen that by combining the special features of various methods together with special purification and sufficient controls the urinary salicyl products appear to be nothing more than ordinary salicylic acid containing traces (more or less) of nitrogen, and not in the form of a-amino-nitrogen. Therefore, they cannot be salicyluric acid.

7. *Roaf's method for the preparation of hippuric acid* (22)

This was tried because of the chemical analogy of salicyluric acid to hippuric acid.

The urine is saturated with one-fourth by weight of ammonium sulphate and sulphuric acid is added. The whole is allowed to stand and gradually crystals separate. These are filtered, and further purified by treatment with hot water and animal charcoal.

One hundred cubic centimeters each of two urine specimens from two different patients (patient 6, experiment 9, and patient 22, experiment 20) containing 0.26 and 0.424 gram salicylic acid, respectively, by the distillation-colorimetric procedure, were treated according to Roaf's method. From the urine of patient 6, long whitish needle-like crystals were obtained with melting point of 156 to 159°. The benzene soluble portion (salicylic acid portion of Stockman) of these crystals had a melting point of 155°; nitrogen by Lassaigne's test was negative. The portion ("salicyluric acid") remaining after washing with benzene had a melting point of 156°; nitrogen absent by Lassaigne's test; iron-salicyl test positive.

The urine of patient 22 yielded thick needle crystals which on further purification had a melting point of 148 to 150°; nitrogen content of 0.79 per cent; iron-salicyl test positive.

8. *Lewinski's method for the extraction of hippuric acid from urine* (23)

This was also tried with the hope of recovering salicyluric acid, the chemical analogue of hippuric acid, from salicyl urine.

One hundred cubic centimeters of urine is evaporated to dryness on the water bath, 2 grams of sodium acid phosphate are

added, together with 15 grams of calcium sulphate, and the whole is triturated to a uniform powder; dried in the oven and percolated with ethyl acetate for two hours. The ethers were allowed to evaporate and the crystalline material left behind was washed with cold water; recrystallized twice from hot water and dried.

In experiment 21, 100 cc. from the same urinary specimen (from patient 22) used for extraction by Roaf's method, and containing 0.424 gram salicyl was used. The crystalline product obtained was very brown, and, after purification, the crystals gave a positive iron-salicyl test; yielded 4 per cent of nitrogen; melting point was 141 to 145°. No further tests were made, for the crystals were too impure and could not be further purified.

In experiment 22, a collection of 30 litres of old standing urines from several different individuals was evaporated and treated according to Lewinski's procedure. Three products were obtained as follows:

A. *Mixed crystals.* The sticky brown material after repeated treatment with charcoal and attempts at recrystallization remained noncrystalline for fifty-three days until it was treated with hydrochloric acid when it promptly precipitated a crop of feathery white crystals. These crystals were further purified and gave a melting point of 154 to 155°; yielded 0.28 per cent of nitrogen and a negative ninhydrin test, while the addition of synthetic salicyluric acid to some of the crystals gave a positive test. The crystals were readily soluble in hot water, alcohol, ethyl acetate, ether, slowly soluble in benzene and chloroform. The supernatant fluid yielded yellowish crystals which on further purification gave a melting point of 154.5 to 156°; 0.395 per cent of nitrogen, and a negative ninhydrin test.

B. *Chloroform soluble or salicylic acid portion.* Before the tests were applied, the crystals were twice purified by treatment with animal charcoal and recrystallization and washing with cold water by centrifugalization. The white feathery crystals obtained had a melting point of 155.5 to 156°; nitrogen content was 0.039 per cent; iron-salicyl test was positive and the solubility was the same as for the crystals in (A).

C. *"Salicyluric acid" portion.* The aqueous portion left after treatment with chloroform was extracted with ether and the crystalline residue left after evaporation of the ether was twice purified by boiling with blood charcoal and recrystallization. The crystals gave a melting point of 155 to 157°; nitrogen content 0.171 per cent; ninhydrin test was negative, while the controls with hippuric acid and synthetic sali-

cyluric acid added to some of the urinary salicyl residue were positive. The solubility of these crystals was about the same as in (A), being perhaps somewhat more readily soluble in benzene and chloroform.

D. The supernatant fluid left from (C) yielded a mixture of white needle-like and brown scaly crystals. Nitrogen content was 3.6 per cent; iron-salicyl test positive; ninhydrin test negative, while control tests with hippuric and synthetic salicyluric acid added to some of the urinary salicyl crystals were positive. The crystals were very slowly soluble in chloroform and benzene, but in other solvents about the same as the crystals in A. B, and C; melting point was 137 to 140°.

The trials made on salicyl urines with the Roaf and Lewinski procedures for hippuric acid did not yield salicyl products that could be characterized as salicyluric acid.

9. Efficiency of various solvents in removing salicyl from urine

It is conceivable that the various immiscible solvents used for the extraction of salicyluric acid do not completely remove the product from urine. This was tested by shaking repeatedly a sample of urine of known salicyl content (by the colorimetric-distillation method) until salicyl-free, and then estimating the aqueous portion by the distillation-colorimetric procedure.

Urine of patient 6 was used. One hundred cubic centimeters in each case containing 0.4 gram salicyl was shaken with fresh portions of solvent for one to two weeks, and until the extracts failed to give a test for salicyl with ferric-alum. The extracts were removed and the remaining urines distilled and the distillates estimated for salicyl. The results are as follows:

(1) After ether = 15.0 mgm. = 3.75 per cent remained unextracted.

(2) After ether = 12.0 mgm. = 3.0 per cent remained unextracted.

(3) After chloroform = 46.3 mgm. = 11.6 per cent remained unextracted.

(4) After petroleum ether = 66.0 mgm. = 16.5 per cent remained unextracted.

(15) After ethyl acetate = 10 mgm. = 2.5 per cent remained unextracted.

It is seen that the most efficient solvents were ether and ethyl acetate, and least efficient was petroleum ether. On the whole, however, the quantities left were so small as compared with the total salicyl present that the unextracted portions remaining are practically negligible, and undoubtedly depend upon the co-efficients of solubility in water and the respective solvents. The quantity retained will be modified by the nature of and the extent of treatment with the solvent. Both ether and ethyl acetate are therefore reliable solvents for salicyl and give as complete an extraction as is practically possible.

These conclusions are based upon the assumption that the salicyl removed by the hydrolytic-distillation-colorimetric method represents all of the salicyl, inclusive of "salicyluric acid." In support of this the following proofs may be cited:

1. Hippuric and synthetic salicyluric acid under the same conditions are easily and completely hydrolized. A different behavior might occur if salicyluric acid is a different conjugation product than hippuric, but there is no good reason to believe that it is different.

2. The acid residues left after distillation in the distillation-colorimetric quantitative method have been repeatedly shaken out with ether, ethyl acetate, chloroform and benzene, and in no single instance has there been a positive iron test for salicyl obtained from such extracts. This has been done with about twenty different urinary specimens.

From all of this it appears, therefore, that ether and ethyl acetate extract practically all of the salicyl contained in urine, and about equally well. It may be doubted if the traces of salicyl left behind represent salicyluric acid. It is more likely that they represent the fraction retained by water in accordance with the law of the distribution co-efficient which governs solubility in water and immiscible solvents.

IV. SYNTHESIS OF SALICYLURIC ACID

The methods that have been previously described are those of Bondi (16) and E. Fischer (17). Bondi's method was used, although Fischer considers his own method superior. It was

impossible for me to obtain the necessary materials for synthesis according to the Fischer procedure.

The object in synthesizing salicyluric acid was to study its physical and chemical properties, and behavior in the organism; also as a control in connection with the identification of the alleged salicyluric acid products from urine.   As a rule the yields which were obtained were small, and at this time the materials necessary for the synthesis are very expensive and practically unobtainable.   In view of the unsuccessful attempts to isolate salicyluric acid from urine, according to the methods described in the fore-part of the paper, salicyluric acid loses pharmacological significance.   However, for those who may be interested, and, also, in order to elucidate the origin of the synthetic salicyluric acid used in my control experiments, a brief description of the synthesis is here presented.

The synthesis proceeds in three distinct stages.   The first is concerned with the preparation of fresh hydrazine hydrate; the second, with the preparation of salicylhydrazide, and the last step, concerns the preparation of the salicyluric acid proper. According to Bondi, the reaction takes place as follows:

$$C_6H_4OH.COOCH_3 + \overbrace{NH_2.NH_2}^{\text{hydrazine}} = C_6H_4.OH.CO.NHNH_2 + CH_3OH$$

$\underbrace{\hspace{3cm}}$ Methyl Salicylate   Salicylhydrazid

$$C_6H_4.OH.CO.NH.NH_2 + NOOH = C_6H_4.OH.CO.N_3 + 2H_2O$$

$$\underbrace{C_6H_4.OH.CO.N_3}_{\text{intermediary product}} + \underbrace{NH_2.CH_2.COOH}_{\text{Glycocoll}} + 2NaOH =$$

$$\underbrace{C_6H_4.OH.CO.NH.CH_2.COONa}_{\text{Sodium salicylurate}} + NaN_3 + 2H_2O$$
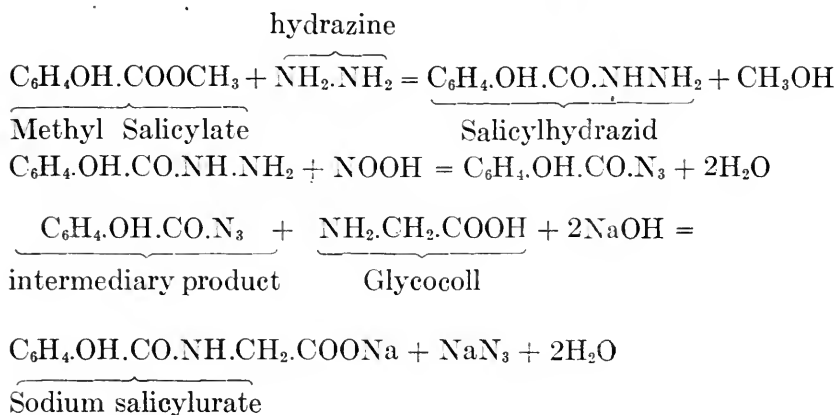
1. Preparation of hydrazine hydrate: This is prepared according to the directions of Lobry de Bruyn (24).

2. Preparation of salicylhydrazid: To the oily residue left after the heating of 10 grams of hydrazine sulphate and 10 grams sodium hy-

droxide, and consisting of hydrazine hydrate, are added 10 cc. of methyl salicylate. The mixture is then heated on a water bath for two hours with a reflux condenser. The flask should not be heated longer than two and one quarter hours, for prolonged heating gives a product with a high melting point. On cooling it forms a hard cake. This is now rubbed in a dish with ether to remove the excess of methyl salicylate; the whole filtered and dried.

The mass is now treated with 25 cc. of 95 per cent alcohol, heated and allowed to recrystallize. A small portion of the crystalline mass does not go into solution, and Bondi ascribes this to the presence of a dihydrazide. From the filtrate a rich mass of crystals is obtained. The product is colorless, pure, and has a melting point of 147°. Bondi quotes Struve and Radenhausen to have observed the melting point of 145°.

After several trials, I found that the quantity of oil of wintergreen recommended is not completely utilized, and, in spite of several washings with ether to remove the excess, an oily sediment would appear when the crystalline mass was dissolved in alcohol. It was necessary to repeat the purification by decanting the alcoholic solution and allow recrystallization from this. In this way, a clean pure white crystalline product free from the ester was obtained which began to melt at 145° and was completely fluid at 147°. This product keeps and may be kept in stock for the next step.

3. Preparation of salicyluric acid: 3 grams of salicyl hydrazide are dissolved in 20 cc. of nitric acid and 50 cc. of water. Twenty cubic centimenters of a sodium nitrite solution are added drop by drop, the mixture being kept cooled in ice and agitating at the same time. After standing for half an hour in ice water the formation of the intermediary acid is completed. The product at this stage has an unpleasant irritating odor. It is filtered and washed with ice water. The moist acid is added to a cooled solution of 1.6 gram of glycocoll in 20 cc. of $\frac{N}{1}$ sodium hydroxide and 50 cc. water. With rapid shaking and the further addition of 23 cc. $\frac{N}{1}$ sodium hydroxide (in portions) solution is completed within an hour.

The liquid is now filtered and the filtrate shaken twice with ether to remove an acid which has now entered solution without altering the reaction. The ether is removed in a current of air. The filtrate is then acidified with sulphuric acid (using Congo red as indicator) and shaken out with ethyl acetate in order to free the reaction product from the action of the acid. From the ethyl acetate extract crystals

gradually separate on long standing, and these belong to a product with high melting point.

The ethyl acetate is separated from the crystals and distilled off to a small volume.   The residue is dried in vacuum.   A colorless crystalline mass is obtained.

To purify, one part of this is dissolved in three parts of hot alcohol. If a portion remains undissolved, it is filtered off, as this is an insoluble by-product.   The alcoholic solution is mixed with 30 cc. of hot benzene and left for a short time connected with reflux condenser on a boiling water bath.   On cooling the flask contains needle-like crystals.   These are dried and a colorless crystalline mass is obtained, which when rubbed is strongly electric.   Bondi states the crystals sinter at 165°, are more completely fluid at 170°, and a clear fluid appears at 171 to 172°.   The product is soluble in ethyl alcohol, methyl alcohol, ethyl acetate and acetone at ordinary room temperature; insoluble in benzene, ether, petroleum ether and chloroform, except when boiling and even then a large volume of solvent is necessary.

In my hands the synthesis proceeded generally according to Bondi's description until the stage of extraction with ethyl acetate was reached.   Several trials were made and this was the usual experience.   The crystals left after evaporation of the ethyl acetate extract were brownish in color.   After treatment with 95 per cent alcohol and benzene, and as the solution became more concentrated on standing, the color became deeper brown. Gradually white and brown colored crystals separated out.   A rather large mass of crystals separated; melting point was 158°. The crystals were now dissolved in hot water and boiled with blood charcoal; filtered and the filtrate allowed to crystallize. Large needle-like crystals separated, and thicker than ordinary salicylic acid.   These were further washed several times with cold water by centrifugalization, and dried.   The product consisted of well-formed large, thick needles (larger than salicylic acid), clean and white.   The crystals glistened at 165°, were practically fluid at 166°, and completely melted at 167°.   This was obtained with two products several times, and the same thermometer with a purified specimen of Kahlbaum's salicylic acid gave 156°.   0.0248 gram of the product yielded 0.00171 gram nitrogen = about 97 per cent of theory; and 0.0284 gram

yielded 0.002 gram nitrogen = 98 per cent of theory. 0.02 gram hydrolyzed with 1 cc. of 10 per cent hydrochloric acid for four hours, then neutralized, gave a brilliant blue color with ninhydrin which, is positive for a-amino acid nitrogen, and was indistinguishable from the blue of a similar quantity of hippuric acid treated in the same way. The crystals were freely soluble in alcohol, ethyl acetate, less readily in ether, hot water and chloroform; very slowly soluble in cold and boiling benzene. The order of efficiency of the solvents beginning with the most efficient was; alcohol, ethyl acetate, ether, hot water, chloroform, boiling benzene, cold benzene, cold water.

The product, therefore, was similar (except for a small difference in melting point) to that prepared by Bondi. This product was used as a control for ninhydrin tests in the experiments described in the fore part of the paper.

V. DISCUSSION

The salicyl products corresponding to both salicylic acid and "salicyluric acid" obtained by the application of various methods to urines under various conditions may be regarded practically the same, that is, salicylic acid of varying purity. The melting points of the products were usually in the neighborhood of 156°C., which corresponds to ordinary salicylic acid. When the crystals were quite impure their melting points were less, but in some cases higher than that of salicylic acid. In a few instances traces of a yellowish residue which should correspond to the so-called salicyluric acid gave positive iron-salicyl tests, but did not melt at 250° to 365°. In two instances white crystalline residues were obtained which did not give positive salicyl tests.

As a rule the yield of the "salicyluric acid" portions was limited and in some cases so small that the tests originally contemplated could not be performed. It appeared as though these small yields, practically traces in some cases, represented unextracted salicylic acid.

It was found difficult to remove all traces of a yellowish impurity in the "salicyluric acid" portions, and apparently also

other impurities. Almost every one of the salicylic acid as well as the "salicyluric" products, when tested, contained nitrogen to a greater or lesser extent, ranging from none to 164 per cent of the theoretic for the "salicyluric acid" portions. These impurities, undoubtedly, are responsible for the variable melting points. In many instances special efforts were made to purify the products in various ways, and almost invariably this resulted in removing or lowering the nitrogen content, and bringing the melting point closer to that of salicylic acid.

So far as the tests for a-amino-nitrogen are concerned these were uniformly negative. They were somewhat questionable with two products only. The tests were always controlled with hippuric acid, and, in addition, synthetic salicyluric acid was added to the urinary salicyl material, in order to exclude any inhibitory influence that the yellowish impurities so commonly encountered might have on the development of the test. Both the hippuric and the synthetic salicyluric acid mixtures always responded positively for a-amino-nitrogen. By this it was conclusively shown that the urinary salicyl products obtained by all of the methods supposed to give "salicyluric acid," did not contain practically any a-amino-nitrogen, and, therefore, no glycocoll, which should theoretically be present in salicyluric acid.

As to solubility, the residues which should correspond to "salicyluric acid" conformed to that reported by various authors for such solvents as alcohol, ethyl acetate, ether, chloroform, benzene, hot and cold water. However, this does not prove that the products were salicyluric acid, particularly if the impurities, which were present, are taken into consideration. Unfortunately some of the "salicyluric" products could not be tested as to solubility because of the very limited yields.

A synthetic salicyluric acid product was prepared according to Bondi's method, and its properties generally agreed with the description of Bondi. This was used as a control with the ninhydrin test for a-amino-nitrogen in the alleged "salicyluric acid" of the urinary extractives.

VI. CONCLUSIONS

The methods which have been hitherto used for isolating what was supposed to be salicyluric acid, did not yield, when applied to salicyl urines, any notable quantity of any pure product. The products that are obtained are not well characterized, do not have any distinctive properties, and apparently do not contain glycocoll.

From this it appears improbable that salicylates are converted into salicyluric acid in the human and animal organism. The products that have been interpreted as "salicyluric acid" were presumably more or less impure salicylic acid, that had been imperfectly separated in the process of isolation.

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A QUANTITATIVE STUDY OF THE EFFECT OF DIGITALIS ON THE HEART OF THE CAT

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The administration of digitalis to patients produces changes in the heart-beat which have been definitely established by means of electrocardiograms. The fact that this drug causes a delay in the time of passage of the cardiac impulse from auricles to ventricles has long been recognized, and its effect on the conductivity of the heart has been extensively studied. The drug is also known to cause the production of premature contractions, which electrocardiograms have shown to arise from ectopic foci either in the auricles or ventricles. The arrythmia thus produced is considered as one of the signs of an excessive dose of digitalis.

Recently Cohn, Fraser and Jamieson (1) have pointed out the almost invariable effect which digitalis has upon the form of the ventricular complex of the electrocardiogram, a change occurring in the so-called T wave, a downwardly directed wave replacing the normally upwardly directed wave (fig. 1, *a* and *c*). The action of the drug in slowing the rate of the heart-beat is one which requires further study, as this effect on the normally beating heart has been recently called into question. Slowing has failed to show itself in clinical observations in which other factors producing slowing have been eliminated (Cohn and Fraser) (2).

The present study was undertaken with the idea of determining experimentally the appearance of these various effects of digitalis action relative to the dose of the drug intravenously injected, and the results are expressed in percentages of the lethal dose. The first series of experiments was carried out

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Fig. 1a. Control Record.

Fig. 1b. Flattening of T wave.

Before injection of digitalis.
2.45 pm. Rate = 220   P−R = 0.062 sec.

30% lethal dose injected
3.15 pm. Rate = 196   P−R = 0.064 sec.

Fig. 1c. Conduction time prolonged.
T wave inverted.

Fig. 1d. Auriculo-Ventricular dissociation
Abnormal V. complexes.

60% lethal dose injected
3.49 pm. Rate = 165   P−R = 0.093

70% lethal dose injected
3.59 pm. Rate = 150 V rate = 152.

Fig. 1e. Varying V. complexes passing into ventricular fibrillation.

100% lethal dose injected
4.26 pm. Varying V complexes and Ventricular Fibrillation.

also with the idea of establishing the usual effect of the drug under the experimental conditions to be described, which would serve for comparison with further series in which the experiments were varied. So far a second series has been carried out in which the vagi were cut before digitalis was administered.

Cats were used in these experiments and digitalis was employed in the form of the tincture, a one to ten dilution with physiological salt solution being injected. The tincture was standardized by the "cat method" described by Hatcher and Brody (3). By this method approximately 1 cc. of the tincture used was found in a series of five animals to be the lethal dose for 1 kilo body weight of cat. This was therefore taken as the minimal lethal dose in determining the dosage used in all experiments.

I. THE EFFECT ON THE HEART WITH THE VAGI INTACT

The first series of ten experiments were carried out as follows: The cats, averaging about 3000 grams in weight, were anaesthetized with ether, a cannula was inserted into the right femoral vein, and electrodes (German silver over bandages soaked in salt solution) were applied to the left hind leg and the right fore leg. The animals were then put in circuit with the string galvanometer, and after control electrocardiograms were obtained (fig. 1, a) the injection of the tincture of digitalis was begun. One-tenth of the calculated lethal dose was administered intravenously every ten minutes. Records were obtained, as a rule, one minute, five minutes and nine minutes after each injection, but the movements of the shadow of the string of the galvanometer were watched constantly, and other records were obtained as soon as any alteration in the movement of the string was observed. Usually about fifty records were taken during the course of each experiment. These records were analyzed to determine changes in the rate of the heart-beat, in the length of the conduction time, in the form of the complexes, and in the type of the cardiac mechanism.

The animals were kept under as light anaesthesia as would allow electrocardiograms to be recorded, and asphyxia was

guarded against. Only those animals which died a typical cardiac death, with one exception, are included in the series. Three animals died before the calculated lethal dose (C. L. D.) was reached. (70, 80, and 85 per cent of the C. L. D.). One died when the C. L. D. had been given, while four required somewhat more than the C. L. D. (106, 120, 139, 140 per cent of the C. L. D.). The cannula became blocked in one experiment when 110 per cent of the C. L. D. had been administered without producing death, while one animal was markedly resistant to the drug and succumbed only after 210 per cent of the C. L. D. had been administered. The results of the experiments have been summarized in two tables. The first table shows the percentage of the actual lethal dose of digitalis which, when injected intravenously, produced the various phenomena that appeared in the records.

TABLE 1.

Vagi intact

NO.	WEIGHT	PERCENTAGE OF ACTUAL LETHAL DOSE CAUSING				
		Inverted T wave	Lengthened P-R time	Idio-ventricular complexes	Con-stant A-V disso-ciation	Slowest rate
	grams.					
1	2483	30.0	50.0 70.0 (Partial block	70.0	83.0	70.0
2	3175	17.0 (First change) 54.0 (Inverted)	69.0	77.0	82.5	82.5
3	3647	24.0	48.0	72.0	84.0	96.0
4	2500	25.0	100.0	87.5	87.5	100.0
5	4340	21.0	36.0	78.0	78.0	61.0
6	3120	20.0 C. L. D.	20.0 C. L. D.	100.0	100.0	50.0
7	3882	28.0 (Flat)	56.0	28.0 (Coupled rhythm)	75.0	66.0
8	2170	30.0	60.0	70.0	70.0	70.0
9	2830	20.0 (Flat)	No change	71.5	71.5	57.0
10	2880	Inverted at onset	34.0 49.0 (Partial block	64.0	69.0	69.0
Average	3103	23.9	52.5	71.6	80.0	72.1

Although the figures of each column show a considerable varia-
tion, they indicate in a general way the dose of digitalis usually
required to produce each effect. The figures show clearly that
the inversion of the T wave of the electrocardiogram, the impair-
ment of auriculo-ventricular conduction and the inception of
auriculo-ventricular dissociation occur independently of one
another and that they represent different stages in the effect
of digitalis on the heart.

The averages of the figures in the various columns indicate
that approximately one-quarter of the minimal lethal dose of
digitalis is necessary to produce the first change in the form of
the T wave. This change consists in a flattening or inversion
of this wave, which is usually upwardly directed in the lead
used (Lead II) (fig. 1, b). Flattening usually progressed to
inversion when more digitalis was administered (fig. 1, c). Ap-
proximately one-half of the lethal dose of digitalis is necessary
to produce a definite prolongation of the P-R interval (fig. 1, c),
and approximately three-quarters of the lethal dose is required
to cause a constant auriculo-ventricular dissociation (fig. 1, d).
The prolongation of the P-R interval was usually gradual rather
than sudden, and it was accompanied by a very gradual slowing
in rate, and both of these phenomena continued until complete
dissociation occurred. Slowing of the heart rate continued until
approximately three-quarters of the lethal dose had been in-
jected, when acceleration set in, and continued until fibrillation
replaced the normal beat. Independent ventricular contrac-
tions, sometimes represented by abnormal complexes, occurred
in five experiments before constant A-V dissociation occurred,
while in the other five these two phenomena occurred with the
same dosage.

The effect of digitalis on the heart rate in these experiments is
shown in table 2, in which is given the rate before the drug was
administered, the slowest rate observed, and the amount of
slowing in beats per minute. The rate of the auricles and of
the ventricles when dissociation first appeared is also given.

The rate of the heart-beat was definitely slowed in all but one
of the experiments, no. 9. If this experiment is excluded, it is

seen that although the amount of slowing is quite variable, the slowest auricular rate in each experiment is strikingly constant, regardless of the initial rate. In all but one of the experiments the ventricles instituted and maintained an independent rhythm before maximum auricular slowing was reached. The ventricular rate was at this time sometimes the same, but more often, slightly higher than the auricular rate.

The figures of the table fail to give a clear idea of the effect which the intravenous injections of digitalis had on the heart

TABLE 2

Vagi intact. Effect of digitalis on the heart rate

NO.	INITIAL RATE	SLOWEST RATE	AMOUNT OF SLOWING	RATES WHEN DISSOCIATION APPEARED	
				Auricular	Ventricular
1	247	134	113	141	124
2	196	110	86	175	175
3	216	137	79	145	200
4	200	137	63	180	180
5	First records lost	123		150	192
6	207	130	77	175	175
7	164	112	52	123	125
8	220	150	70	150	152
9	220	(200)	20	218	218
10	164	131	33	133	150
Average	203.5 beats	129*	65.9 beats	159	169.1

*No. 9 omited.

rate. Except in experiment 9, there was always a gradual slowing of the auricular rate usually until or after auriculo-ventricular dissociation set in. After the dissociation the ventricular rate gradually increased. In one experiment the auricular rate increased before the dissociation occurred, but usually auricular acceleration did not take place until after the dissociation, and it was as a general rule less marked than the ventricular acceleration.

The independent ventricular activity always showed itself by yielding complexes differing distinctly from those of the

sequential beats.   They were at first larger complexes, diphasic in character, usually with the initial wave upwardly directed and quite high.   As the experiments progressed, the type of the ventricular complexes often altered so that the initial wave was downwardly directed, and later on a variety of rapidly recurring complexes appeared.   The cardiac activity yielding this type of record was soon superseded by ventricular fibrillation which marked the end of the experiment in eight instances (fig. 1, e).   In one experiment the electrocardiograms at the end were not characteristic of fibrillation, while in another, no. 6, death was not produced by digitalis, on account of blocking of the cannula.

The effect of digitalis on the ventricles is worthy of further notice.   The drug appears to increase the rhythmicity of the ventricles while the auricles are being slowed, so that at some time in the experiment the rhythmicity of a focus in the ventricles exceeded that of the auricular pace-maker and dissociation resulted.   The same effect is apparently taking place in the auricles, and it seems to oppose the slowing effect, as at some time in the experiment acceleration begins, usually later than that of the ventricles and to a less extent.

When the ventricles first contracted independently of the auricles, in several experiments the point of origin of the impulse, as judged by the form of the complex, was in the right ventricle. Later on the point of origin of the impulse shifted to another part of the heart, and the form of the complexes changed, while later still impulses arose from several points.   This was followed by ventricular fibrillation.   The fact that the ventricular rate exceeded that of the auricles after dissociation set in, suggests that the mechanism causing slowing is less effective on the ventricles than on the auricles, a result to be expected if vagus stimulation is responsible for the auricular slowing.

The usual effects of digitalis upon the human heart as shown by the electrocardiographic method have been already briefly considered.   These effects may be compared with those observed in the present experimental study.   Of course only those effects which appear after small percentages of the lethal dose

in the animals could be expected to occur in patients who receive therapeutic doses. Perhaps the most constant phenomenon found in both is the inversion of the T wave which occurs in cats when between 20 to 30 per cent of the lethal dose has been given. We do not as a rule give much more than sufficient digitalis to produce this effect in man and consequently the other effects produced should correspond roughly to those observed after 30 per cent or at most 40 per cent of the lethal dose in animals. With this dosage the cat's heart shows a slowing in rate which amounts to only about 10 per cent of the initial rate, a change which might not attract attention in patients under ordinary clinical conditions. Very much more marked changes in conduction frequently occur in patients than those observed experimentally with small doses of the drug, and this no doubt is due to the fact that in many individuals who receive digitalis the conductivity of the junctional tissues is already impaired. It is worthy of note that bigeminy and ventricular escape, both of which are observed in patients, usually occurred only after large doses of the drug had been administered to the animals in these experiments. The protocol of experiment 8 is given as typical of the others, and several of the electrocardiograms from this experiment are shown in figure 1.

Summary

A series of ten experiments is reported in which the tincture of digitalis was administered intravenously to cats in a series of doses while records of the heart beat were being obtained by electrocardiograms. An analysis of these records shows that a change in the form of the ventricular complex, the inversion of the T wave, is the first constant sign of the digitalis action. This occurs approximately when 25 per cent of the minimal lethal dose has been given.

Prolongation of the conduction time between auricles and ventricles first occurs with doses which vary considerably in different animals. The doses causing this effect average approximately 50 per cent of the minimal lethal dose. Idio-ventricu-

lar complexes occur in the records when approximately 70 per cent of the minimal lethal dose has been given, and constant auriculo-ventricular dissociation occurs with about 80 per cent.

The heart rate of the cat is slowed by the intravenous injection of digitalis. This occurs gradually and becomes definitely apparent when about 25 per cent of the minimal lethal dose has been given. Maximum auricular slowing usually occurs with about 70 per cent of the mimimal lethal dose, and the minimal rate is fairly constant, regardless of the initial rate. Auricular acceleration occurs with further administration of the drug, but during the latter part of the experiments the independently beating ventricles show greater acceleration. The drug almost invariably produces finally ventricular fibrillation, cardiac death resulting.

II. THE EFFECT AFTER THE VAGI HAVE BEEN CUT

A series of eight experiments have been performed in which the procedures were the same as those already described except that after a constant record had been obtained both vagus nerves were cut in the neck. Before the intravenous injection of the digitalis, each vagus was stimulated by a faradic current, and the records show that this procedure produced the usual results in all experiments. The dosage of digitalis was the same as before, one-tenth of the calculated minimal lethal dose being injected every ten minutes, the tincture of digitalis being diluted ten times with physiological salt solution.

Of this series three animals died before the calculated lethal dose was reached (70, 84 and 95 per cent of the C. L. D.). The other five animals all required one extra dose after the calculated lethal dose had been given, and died when 110 per cent of the calculated lethal dose had been administered.

A comparison of this series of experiments with the first series when the vagi were intact shows that no striking change in the toxicity is caused by cutting the nerves. The series are too small from which to draw definite conclusions on this point. The minimal lethal dose of digitalis was more uniform and nearer the calcu-

PROTOCOL OF EXPERIMENT 8

Cat 16; Weight 2170 grams; calculated lethal dose of diluted tincture, 220 cc. Date: March 6, 1917

CURVE	TIME	AMOUNT cc.	TIME OF INJECTION	LETHAL DOSE per cent	P-R TIME SEC.	RATE PER MINUTE	CARDIAC MECHANISM	FORM OF ELECTROCARDIOGRAM
Fig. 1, a 1...	2.45	0		0	0.062	220	Normal	P = 2 mm., R = 4 mm., T = 1 mm. positive
2...	2.56	2.2 (2.53)		10	0.063	212	Normal	No change
3...	3.02½	2.2 (2.53)		10	0.064	204	Normal	No change
4...	3.05	4.4 (3.04)		20	0.059	205	Normal	No change
5...	3.09	4.4 (3.04)		20	0.063	202	Normal	T slightly less well defined
6...	3.12	4.4 (3.04)		20	0.057	203	Normal	T slightly less well defined
Fig. 1, b 7...	3.15	6.6 (3.14)		30	0.069	196	Normal	T flattened slightly. R not so sharp
8...	3.19	6.6 (3.14)		30	0.062	190	Normal	T wave inverted. R = 5 mm.
9...	3.23	6.6 (3.14)		30	0.063	191	Normal	T wave more extensively inverted
10...	3.25	8.8 (3.24)		40	0.064	185	Normal	No change
11...	3.30	8.8 (3.24)		40	0.063	196	Normal	T wave deeper
12...	3.32½	8.8 (3.24)		40	0.068	185	Normal	No change
13...	3.35	11.0 (3.34)		50	0.066	183	Normal	T wave not so definite, sometimes absent.
14...	3.40	11.0 (3.34)		50	0.067	171	Normal	T wave less definite
Fig. 1, c 15...	3.43	11.0 (3.34)		50	0.067	178	Normal	T wave less definite
16...	3.45	13.2 (3.44)		60	0.077	170	Normal	Descent of R less abrupt
17...	3.49	13.2 (3.44)		60	0.083	165	Normal	T wave more definitely inverted
18...	3.53	13.2 (3.44)		60	0.074	164	Normal	No change
19...	3.55	15.4 (3.54)		70	0.074	156	Normal	No change

Fig. 1, d 20...	3.59	15.4 (3.54)	70	A = 150 V = 152	A-V dissociation	V complexes entirely different; upwardly directed
21...	4.00	15.4 (3.54)	70	A = 177 V = 177	A-V dissociation	No change
22...	4.02	15.4 (3.54)	70	A = 158 V = 158	A-V dissociation	V complexes vary
23...	4.04	17.6 (4.04)	80	A = - V = 200	A-V dissociation	P not seen; V complexes different
24...	4.05	17.6 (4.04)	80	A = 176 V = 200	A-V dissociation	V complexes all the same
25...	4.06	17.6 (4.04)	80	A = 169 V = 195	A-V dissociation	No change
26...	40.8	17.6 (4.04)	80	A = 171 V = 205	A-V dissociation	New type of V complex; downwardly directed
27...	4.09	17.6 (4.04)	80	A = 175 V = 209	A-V dissociation	V complexes vary; upwardly directed
28...	4.11	17.6 (4.04)	80	A = 158 V = 212	A-V dissociation	No change
29...	4.13	17.6 (4.04)	80	A = 180 V = 216	A-V dissociation	No change
30...	4.15½	19.8 (4.15)	90	A = 186 V = 225	A-V dissociation	No change
31...	4.18	19.8 (4.15)	90	A = 200 V = 233	A-V dissociation	No change
32...	4.19	19.8 (4.15)	90	A = ? V = 240	A-V dissociation	Complexes vary
33...	4.19¼	19.8 (4.15)	90	A = 205 V = 232	A-V dissociation	Complexes vary a few sequental beats
34...	4.20	19.8 (4.15)	90	A = 236 V = 236	A-V dissociation	Complexes downwardly directed
35...	4.21	19.8 (4.15)	90	A = 237 V = 237	A-V dissociation	No change
36...	4.22	19.8 (4.15)	90	A = 243 V = 243	A-V dissociation	Complexes vary
37...	4.22½	19.8 (4.15)	90	A = ? V = 246	A-V dissociation	V complexes vary
38...	4.23	19.8 (4.15)	90	A = ? V = 246	A-V dissociation	No change
39...	4.24	19.8 (4.15)	90	V = 247	A-V dissociation	V complexes more irregular
40...	4.25¼	22.0 (4.25)	100	V = 244	A-V dissociation	Marked variations in V complex
41...	4.26	22.0 (4.25)	100			Fibrillation of ventricles
42...	4.27	22.0 (4.25)	100			Marked variation in F complexes passing into fib
Fig. 1, e 43...	4.28	22.0 (4.25)	100			Recovery from V Fib.; marked irregularities
44...	4.30	22.0 (4.25)	100			Fibrillation alternating with periods of recovery
45...	4.30½	22.0 (4.25)	100			Slow V fib. Large irregular waves
46...	4.30¾	22.0 (4.25)	100			Auricles beating without ventricles

lated lethal dose after the vagi were cut, and none of the animals showed the relative tolerance to the drug that was observed in three or four of the cats of the first series. These results appear to agree with those of the larger series of experiments which Macht and Colson (4) have performed, of which only a brief report is as yet available. They found that the minimal lethal dose was much more uniform for vagotomized cats than for those with the vagi intact. They also found that the toxicity of digitalis bodies for vagotomized cats was greater than that for non-vagotomized animals.

The control curves taken before and after the vagi were cut indicate that this procedure caused no change in the form of the electrocardiograms.

The results of this series of experiments in which the vagi were cut have been analyzed similarly to those of the first series, and are presented in the following tables.

TABLE 3

Vagi cut

NO.	WEIGHT	PERCENTAGE OF LETHAL DOSE CAUSING				
		Altered T	Longthened P-R	Idio-ventric-ular	A-V disso-ciation	Slowest rate
	grams					
1	2780	20.9	81.8	91.0	91.0	54.6
2	3030	45.5	0	81.9	81.97	72.8
		(Inversion)				
3	1960	27.3	0	81.9	81.9	45.5
4	2875	25.0	0	87.5	87.5	62.5
5	2000	44.4	80.0	100.0	100.0	17.8
			Rate 300+			
6	3680	27.3	63.6	72.7	72.7	63.6
7	2480	30.0	0	80.0	80.0	30.0
8	2200	Inversion at onset		60.0	60.0	10.0
Average........	2626	31.5		82.1	82.1	44.6

In table 3, which deals with the relation of certain phenomena observed in the records to the percentage of the lethal dose of digitalis injected intravenously, it is seen that the T wave of the ventricular complex is flattened or inverted with about 30

per cent of the lethal dose, approximately the same amount of digitalis as when the vagi are intact. In two experiments this change occurred unusually late and so the average is higher, but the figures of tables 1 and 3 are distinctly comparable in this regard. In those cases in which flattening occurred as the first change in the T wave, the change progressed to inversion in all cases. In one other respect the heart was affected by the drug apparently in the same manner before and after cutting the vagi. Auriculo-ventricular dissociation occurred with practically the same percentage of the lethal dose of digitalis. This seems to indicate that the increase in the idio-ventricular rate which the drug invariably produced was caused independently of its action through the vagi. The onset of auriculo-ventricular dissociation occurred only after large amounts of the drug had been given, and represents a rather late toxic effect. The dissociation was brought about in these experiments independently of auricular slowing, which was a factor in its production when the vagi were intact. This difference in the two series is brought out when the effect of the drug on the heart rate is considered, as will be seen below.

Except for the effect on the T wave of the electrocardiogram and on the ventricular rate, the effects of digitalis on the heart were distinctly altered by cutting the vagi. The A-V conduction was not altered with the same constancy or degree as when the vagi were intact. In four out of seven experiments no delay in the conduction time occurred. In two experiments definite prolongation suddenly appeared after large percentages of the drug had been given, and in a third experiment the conduction time became suddenly prolonged a few minutes before auriculo-ventricular dissociation set in when 65.6 per cent of the lethal dose had been injected. In one experiment the auricles and ventricles were beating independently from the onset of the experiment, so that the conduction time could not be measured.

The change in the auriculo-ventricular conduction time that occurred in the three experiments of this series may be interpreted as effects of the drug directly on the junctional tissues or

as evidence of damaged conduction as one of the phenomena of impending death of the heart.

Independent ventricular contractions as indicated by idio-ventricular complexes in the electrocardiograms did not occur as early in the experiments when the vagi were cut as they did with the nerves intact, and they appeared only when auriculo-ventricular dissociation set in in every instance. This result indicates that digitalis tends to cause idio-ventricular beats or extrasystoles at least in part indirectly through its action on the vagi.

The effect of digitalis on the heart rate is summarized in table 4.

TABLE 4

Vagi cut.   Effect on rate

NO.	INITIAL RATE	AFTER VAGI CUT	SLOWEST A RATE	AMOUNT OF SLOWING AFTER VAGI WERE CUT	DISSOCIATED RATES	
					Auricular	Ventricular
1	253	266	193	73	260	250
2	171	260	212	48	227	227
3	216	242	184	58	?	266
4	262	286	237	49	245	245
5	257	262	220	42	?	284
6	227	228	178	50	?	225
7	255	270	222	48	262	262
8	192	224	184	27	224	212
Average........	229	253	203.8	49.4	243.6	246.4

The figures of the table show that the effect of the drug on the heart rate is distinctly altered when the vagi are cut. It is true that a moderate amount of slowing occurred during the administration of digitalis, especially in the early part of the experiments, but the constant gradual slowing that was observed in the first experiments, as illustrated by the protocol of experiment 8, did not take place. The tables do not bring out clearly this difference between the two sets of experiments, but when all the protocols are reviewed, a striking difference in the effect on the heart rate is seen between the animals with the vagi cut and those with the vagi intact. In the second series slowing of the auricles was never an important factor in the production of

auriculo-ventricular dissociation, and in all instances the auricular rate was relatively high when dissociation occurred. The ventricular rate was also constantly higher at this time than in the first series.

Two control experiments were done to determine whether changes in rate or in the A-V conduction time would take place without digitalis if cats were etherized and the vagi cut. The animals were kept under observation for about two hours, electrocardiograms being taken at frequent intervals. No change in the A-V conduction time occurred in either experiment. In the first experiment the rate varied between 187 and 204 beats per minute, and in the second between 247 and 185 per minute, the initial rate being the fastest observed. No progressive or constant slowing was observed, but it must be noted that the change of rate of 58 beats was exceeded in only one experiment in the second series, while it was exceeded in six of the first series of nine experiments in which the amount of slowing could be calculated.

The results of the experiments in which the vagi were cut indicate that the change in the form of the T wave of the electrocardiogram is independent of the action which digitalis exerts indirectly on the heart through the vagi. This change in the T wave may be considered therefore as a direct action on the heart muscle. This result is in harmony with the findings of Cohn, Fraser and Jamieson (1), who found that in man the injection of atropin did not cause the disappearance of the alteration in the T wave which digitalis had produced. The increase in the idio-ventricular rate as shown by the ventricular rate when A-V dissociation occurred is also apparently due to the direct action of digitalis on the heart, as cutting the vagi had no definite effect upon its occurrence. The ventricular rate was higher at the time of dissociation in the second series, but the ventricular rate rapidly increased in the first series after A-V dissociation, and it was apparently the relatively slow rate of the auricles in the first series that was responsible for the slower ventricular rate at which dissociation took place. A-V dissociation takes place whenever some point in the ventricles generates impulses at a rate higher than that of the auricles.

Our experiments demonstrate conclusively that digitalis acts on the heart of the cat in part directly and in part through the vagi. The prolongation of the A-V conduction time and the slowing of the heart rate which occurred practically constantly when the vagi were intact did not occur when these nerves were cut, with the exceptions that have been described. These effects are almost entirely caused by the action of the drug on the vagus center, a fact that has been long known regarding the effect of the drug on heart rate.

The moderate slowing that occurred in the vagotomized cats may have been caused by the action of digitalis on the peripheral end of the vagi. Such action has been described by Kockmann (5) who found that the injection of atropin caused an acceleration of the heart rate in digitalized animals after the vagi were cut. This peripheral action of the drug has been denied by Etienne (6) who did not however employ atropin in his experiments, and the experiments of Kockmann are the more convincing on this point. The experimental methods of both investigators are to be criticized in the light of more recent work, as both gave preliminary injections of morphine, a drug which has been shown by electrocardiographic methods by Einthoven and Wieringa (7), Cohn (8) and others to be a potent vagus stimulant.

Summary

The experiments indicate that the action which digitalis exerts on the cat's heart is altered when the vagi are cut. The inversion of the T wave occurs practically with the same dosage and to the same extent as it does when the vagi are intact, but the auriculo-ventricular conduction and the heart rate are almost unaffected by digitalis intravenously injected to the lethal dose after the vagi have been cut. These findings indicate that the drug exerts its action on the heart of the cat both directly and through its effects on the vagus center.

The direct action on the heart as shown by the change in the T wave of the electrocardiogram is the first definite effect to appear when a constant percentage of the lethal dose of the drug is injected at intervals intravenously.

The moderate slowing of the heart rate which followed the injection of digitalis in the vagotomized cats may be an indication of the stimulation of the peripheral end of the vagi.

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ON THE ABSORPTION OF DRUGS AND POISONS THROUGH THE VAGINA

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The vagina is generally regarded as an organ incapable of absorbing pharmacological agents. Witness to this widespread belief is the universal and indiscriminate employment by women of all kinds of medicaments—some known to be innocuous and others recognized, otherwise, as violent poisons—in the form of douches, tampons, suppositories, "uterine wafers," etc. Such a practice is not only condoned by the majority of medical men but is often deliberately recommended by them, the prevalent opinion among the profession being that drugs applied to the vagina exert only a *local* effect and are not absorbed into the system. Indeed some clinicians doubt that even opium or belladonna administered per vaginam are capable of producing their characteristic narcotic or anti-spasmodic effects and apply those remedies solely as local or topical reagents. The histological structure of the vaginal wall itself—with its three distinct layers of connective tissue, muscularis, and mucosa, the latter consisting of numerous layers of stratified epithelium and closely resembling the skin except for the absence of the horny layer (1)—would certainly a priori tend to support this view. It is therefore not surprising that while cases of poisoning through the vagina are occasionally reported, such cases are considered as exceptional or are commonly attributed to other causes, such as absorption through a puerperal uterus.

The question of absorption of chemical substances through the vagina has up to the present not been definitely settled. No experimental work on lower animals has, as far as the author knows, been done on the subject. The only experimental work on record comes from two clinicians, Hamburger (2) and Menges (3). These two authors, experimenting on patients, noted the absorption into the system of a number of non-toxic medicaments. As such experimentation is, however, for obvious reasons, limited and impracticable, further investigation of the subject was deemed very desirable. In connection with a study of the absorption of drugs through devious and unusual channels carried on by the present author, the results of which have in part been already published and in part are to appear in due time, a systematic investigation of the absorption of drugs and poisons through the vaginal wall has been undertaken. The results of this work have turned out to be of such general interest and practical importance that it was found desirable to report them in this place.

METHOD

Most of the experiments were made with cats and dogs. The dog was found to be by far the most suitable animal for the purpose, as the structure of its vagina is histologically practically the same as that of the human being (4), and furthermore because in experimenting on that animal the absorption of the substances studied through the uterine cavity can be easily avoided. The drugs studied were, firstly, a series of alkaloids; secondly, a number of inorganic salts, and lastly, some of the more common antiseptics. Evidence of the absorption or non-absorption of the bodies studied was of a two-fold character. In some cases the pharmaco-dynamic and toxicological effects were taken as positive proofs of the constitutional action of the reagents; in other cases the substances after absorption were identified through chemical methods; and in still other cases both physiological and chemical evidence could be abundantly obtained.

ABSORPTION OF SOME ALKALOIDS

Apomorphin. In a paper on the absorption of apomorphin and morphin through the conjunctiva, which appeared elsewhere (5), the present author has shown that apomorphin furnishes a very convenient means of demonstrating the absorption of drugs through the eye. A small dose of that alkaloid either in the form of powder or in solution instilled into the conjuctival sac promptly produces vomiting. Inasmuch as apomorphin is perhaps the best example of emetics acting by the direct stimulation of the vomiting centre in the medulla, this observation points to its absorption through the conjunctiva. Further work by the present author, reported elsewhere, (5b), has shown that the absorption of apomorphin in this experiment is not due to its indirect passage through the nasal duct into the pharynx but that the alkaloid is absorbed, at least in part, through the blood and lymph channels.

Absorption of apomorphin through the vaginal walls can be demonstrated in exactly the same way. A small quantity of apomorphin introduced into the vagina was found to induce vomiting in about five minutes. The following protocol will serve as an illustration.

Experiment June 11, 1917. Black and white dog, 8 kilos. Introduced into vagina 10 cc. of a 1 per cent solution of apomorphin, very slowly and without exerting much pressure. Vomiting follows in four minutes.

Morphin. Inasmuch as the vomiting center of dogs is almost as sensitive to the effects of morphin as to that of its derivative, apomorphin, administration of morphin through the vagina was found to induce vomiting in very much the same way. A little morphin solution introduced in this manner was followed by repeated vomiting in a few minutes. The following protocol will serve as an illustration.

Experiment June 12, 1917. Black and white dog, 8 kilos. Introduced into vagina 10 cc. of a 1 per cent morphin solution. Vomiting follows in five minutes.

Pilocarpin. The characteristic salivation following the exhibition of pilocarpin by administration through the mouth or through subcutaneous injections was found to appear also after the introduction of a few milligrams of this alkaloid into the vaginal canal. Thus on instilling 5 cc. of a 1 per cent solution pilocarpin hydrochloride, distinct salivation was noted after the lapse of half an hour.

Atropin. In experimenting with this alkaloid, its absorption could be conveniently demonstrated by observations of its effect on the heart and circulation. As is well known, a small dose of atropin injected into a dog produces paralysis of the terminals of the vagi nerves in the heart wall, the result being a supression of the normal cardio-inhibitory impulses and a consequent acceleration of the heart beat. Furthermore, a stimulation of the vagi nerves themselves after such an administration of atropin fails to produce the usual inhibition or stoppage of the heart action. Such an effect can be very easily demonstrated after the introduction of a few milligrams of atropin into the vaginal canal, as is well illustrated by the following protocol.

Experiment 15. Dog 6 kilos, narcotized with paraldehyde. Normal heart beat 80 per minute. Amount of current required to produce complete inhibition of the heart, 14 Kronecker units or 360 C.G.S. units.

Introduced 5 cc. of a 1 per cent atropin solution into the vagina.

Three minutes later heart rate 100 per minute; amount of current required to produce inhibition, 46 Kronecker units or 1019 C.G.S. units.

Five minutes later amount of current required to produce inhibition, 280 Kronecker units or 5451 C.G.S. units.

Eight minutes later, no inhibition produced even by much greater stimulation.

Cocaine. As an index of absorption of this alkaloid, just as in the case of atropin, its effect upon the blood pressure and respiration were noted. It was found that a few minutes after the introduction of a few cubic centimeters of a 5 per cent solution of cocaine into the vagina, the blood pressure curve was distinctly affected. Thus, for instance, in one experiment,

six minutes after the administration of the drug, the pressure fell; fifty minutes later the animal went into convulsions, the convulsions being followed by a marked rise in pressure. A distinct effect on the respiration was also noted, the breathing being at first stimulated and later paralyzed.

Aconitin. This alkaloid which is known to pharmacologists as perhaps our most powerful alkaloidal poison, a few milli-
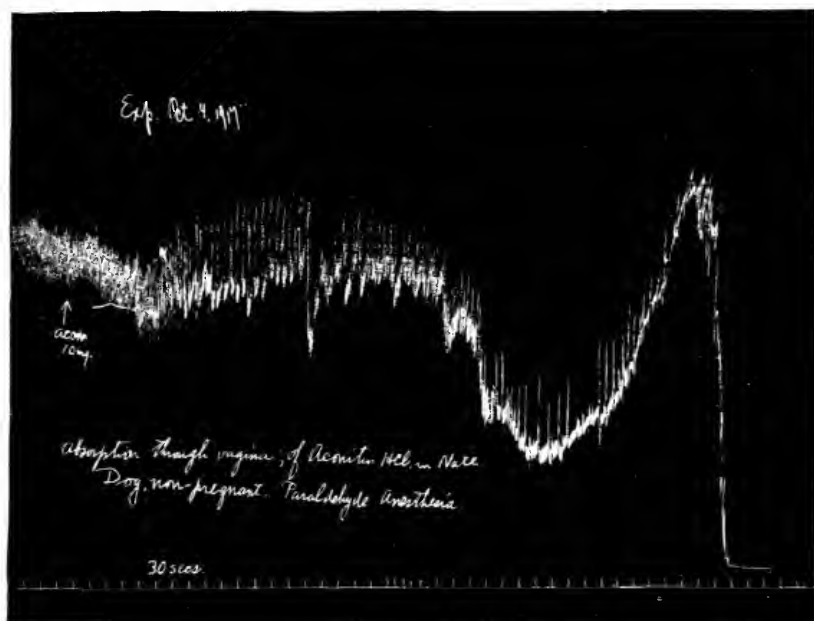
FIG. 1. ABSORPTION OF ACONITIN HYDROCHLORIDE THROUGH NORMAL VAGINA
OF A NON-PREGNANT DOG

grams being sufficient to produce toxic and even fatal effects in a large animal, gave one of the most striking proofs of the absorption of poisons through the vagina. A few milligrams of aconitin hydrochloride introduced into the vagina showed definite signs of absorption after the lapse of two or three minutes, as could be demonstrated by the study of blood pressure and respiratory curves. Kymographic tracings showed a very marked effect upon the heart. The blood pressure ex-

hibited a primary fall, followed by a very striking vagus inhi-
bition of the heart; this was quickly followed by a very
irregular heart-action with wide fluctuations in the blood
pressure curve and a final drop to the death level. The respira-
tion was also seen to exhibit typical inhibition and paralysis
characteristic of aconitin poisoning. Death from administra-
tion of aconitin per vaginam generally followed in less than ten
minutes. Figure 1 is a good illustration of aconitin poisoning
through the vagina.

ABSORPTION OF SOME SALTS.

In order to determine the absorbability of dissociable salts
through the vaginal walls, experiments were made with aquaeous
solutions of potassium iodide, potassium ferrocyanide and po-
tassium cyanide. The results obtained were so striking and
conclusive that experimentation with other salts was deemed
superflous and unnecessary.

Absorption of potassium iodide. On introduction of a few
cubic centimeters of a saturated solution of potassium iodide
into the vagina, positive proof of its absorption could be demon-
strated by examinations of catheterized specimens of urine.
One hour after the introduction of the drug, the urine was found
to give a distinct chemical reaction for iodine. The test em-
ployed was as follows: A few cubic centimeters of the urine
were treated with 0.5 cc. of dilute sulphuric acid; 0.5 cc. of a 2
per cent sodium nitrate solution and 1 cc. of chloroform were
then added and the mixture shaken. A pink color indicated
the presence of the drug.

Absorption of potassium ferrocyanide. The absorption of this
salt could be even more strikingly demonstrated than in the
case of potassium iodide. A tampon soaked with a 5 per cent
solution of the salt and introduced into the vagina produced
an intense Prussian blue reaction in a catheterized specimen
of urine when treated with ferric-chloride one hour after the
administration of the drug. In this place it is well to note that
absorption of potassium iodide and potassium ferrocyanide

through the vagina has also been demonstrated by Hamburger in the human being.

Absorption of potassium cyanide. This poison though of no practical value in therapeutics, beautifully illustrates the absorption of poisons through the vaginal walls, as may be graphically seen from figure 2. Five cubic centimeters of a 1 per
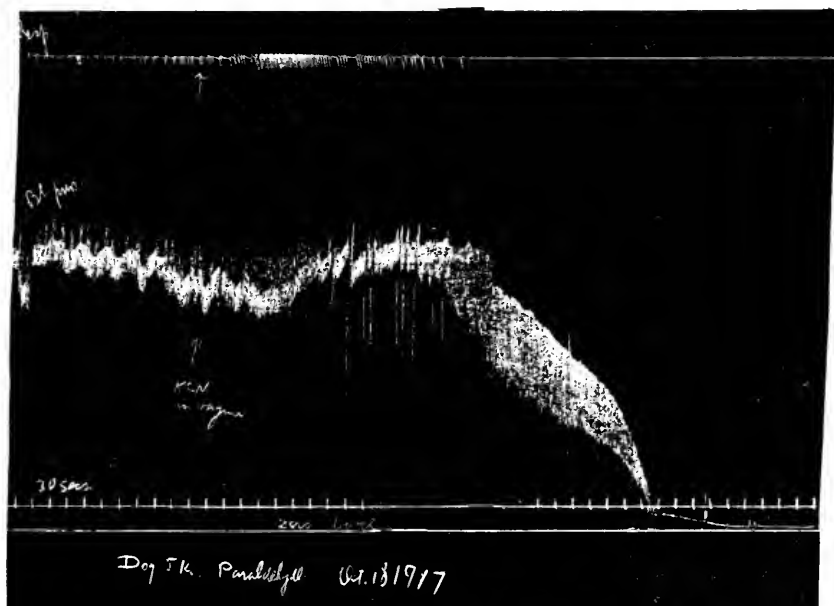
FIG. 2. SHOWING ABSORPTION OF 1 PER CENT SOLUTION OF KCN FROM THE VAGINA OF A NON PREGNANT DOG

cent solution of KCN slowly injected into the vagina was found to show signs of absorption in a very few minutes, as may be seen from the figure. The effect of potassium cyanide on the respiration and on the heart action is very prettily illustrated in this record. It will be seen that the respiration is paralyzed long before the heart ceases to beat.

ABSORPTION OF NITROGLYCERINE

The absorption of glyceryl nitrate through the vagina can be very easily demonstrated, as the author has repeatedly noted. As the Spirits of Glonoin contains a large percentage of alcohol and as this ester is known to be absorbed through mucous membranes and even through the skin, its absorption through the vagina cannot however be regarded as surprising or unusual.

ABSORPTION OF SOME ANTISEPTICS

An investigation as to the absorbability of some of the commoner antiseptics through the vagina was deemed especially desirable as these substances are widely used in vaginal douches and as some cases of poisoning following such use by women have from time to time been reported. The present author made observations on the effects of phenol, cresol and mercuric chloride.

Absorption of phenol. That phenol or carbolic acid is easily absorbed through the vaginal walls was demonstrated by the author by studies of blood pressure and respiratory curves. On introducing even weak solutions (2 per cent) of carbolic acid into the vagina, a distinct fall in blood pressure and impairment of the respiration was noted. Figure 3 illustrates such an action produced by irrigation of a dog's vagina with a 2 per cent solution of phenol for five minutes. The toxic effect of the drug in this case was removed by a subsequent irrigation with normal saline.

Absorption of cresol. A 5 per cent solution of Liquor Cresolis Compositus (U. S. P.) was found to be absorbed in very much the same way as phenol, producing changes in pressure and respiratory curves. A quantitative comparison of carbolic acid and cresol was not made.

ABSORPTION OF CORROSIVE SUBLIMATE

In order to detect the absorption of mercuric chloride through the vaginal walls, the author took advantage of both its physio-

logical and toxicological action on the one hand, and of its chemical reactions on the other.   The poison was administered
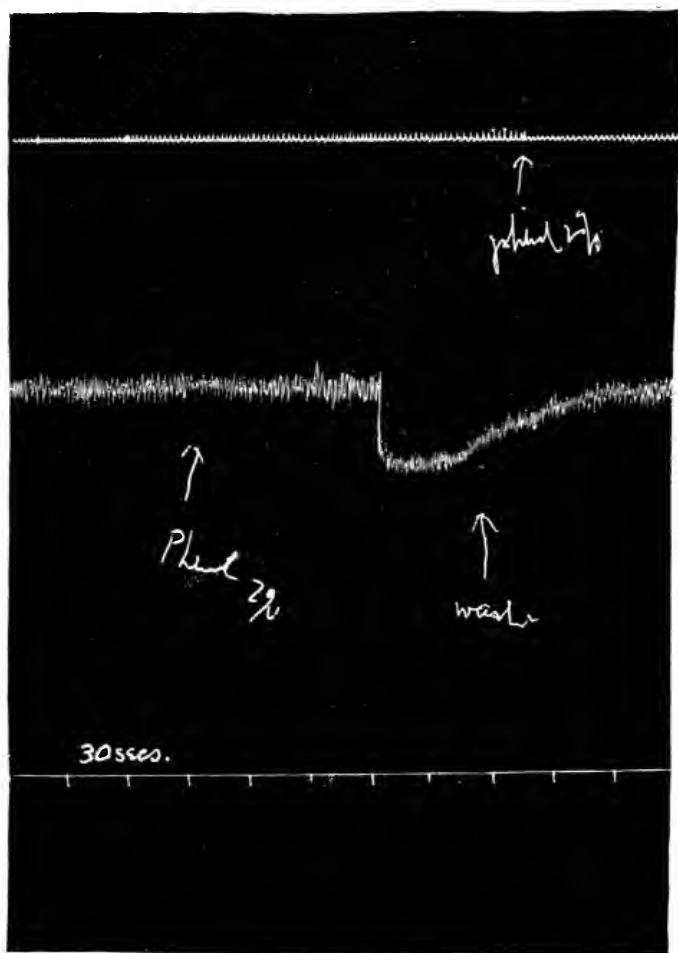
FIG. 3. SHOWING ABSORPTION OF 2 PER CENT CARBOLIC SOLUTION FROM VAGINA
OF NON-PREGNANT DOG

both in the solid state in the form of ordinary antiseptic tablets and in solution.   In both cases corrosive sublimate was found to be easily absorbed through the vaginal walls.   Thus, for in-

stance, the introduction of a fraction of a tablet (about 3 grains) of mercuric chloride into the vagina of a large dog was followed an hour later by marked salivation and violent diarrhea, the animal dying about four hours later. Other dogs in similar experiments developed signs of slower absorption and succumbed in the course of a few days with lesions of typical acute nephritis.

That even smaller doses of bichloride of mercury administered in solution either by tampons or through douches are easily absorbed could be demonstrated by chemical examinations of catheterized specimens of urine. Thus, for instance, a tampon soaked in 1 : 1000 solution of bichloride and introduced into the vagina of a large cat showed distinct traces of mercury in the catheterized urine the following day. The test for mercury in the urine employed in the experiment was as follows: The specimen was first treated with potassium chlorate and strong hydrochloric acid and the mixture heated on a water bath until all traces of chlorine were gone. The mercury was then precipitated on copper wire or coil and identified.

DISCUSSION

From the above described experiments and investigation, it is evident that all kinds of pharmacological agents—alkaloids, inorganic salts, esters and antiseptics—can be and are comparatively easily absorbed through the vaginal walls. These facts are, it is hardly necessary to emphasize, of great therapeutical and toxicological importance. Thus from the therapeutic point of view they indicate that drugs such as opium or belladonna may be rationally administered for their constitutional effects through the vaginal route. On the other hand, even of greater importance is the toxicological evidence furnished by the absorption of some of the substances studied. The prevalent opinion that medicaments administered through vaginal douches, tampons and other forms of treatment exert only a local action has undoubtedly been responsible for a great many cases of dangerous poisoning and may be the explana-

tion of a still greater number of chronic intoxications of an indefinite character. A review of the literature in fact reveals a larger number of poisonings and intoxications through the vagina than is generally supposed and in many of those cases the possibility of explaining the absorption of the drug not through the vagina but through the uterine cavity can be positively excluded.

<div align="center">REVIEW OF CLINICAL EVIDENCE</div>

A search through the medical literature of all languages brings out in a striking way the conclusions just emphasized. A brief review of some of the cases reported may be of interest.

Heberda (6) reports an acute case of arsenical poisoning with suicidal intent in the case of a woman who introduced some solid arsenic into her vagina and died in two days. The same author cites five other similar cases. Brisken (7) reported a case of arsenical poisoning resulting from an attempted abortion. Other cases of arsenical poisoning are recorded by Justow (8) and Ansiaux (9). The latter author relates two cases of arsenical poisoning with homicidal intent perpetrated by an Italian. The criminal administered arsenic to his wife through the vagina in order to get rid of her and, having married a second time, repeated the same procedure when he became dissatisfied with his second. Dewar (10) reports an interesting case of belladonna poisoning through the vagina. Minich (11) describes a case of poisoning by zinc sulphate after local application to the vagina. Schwarz (12), Harnack (13) and others have described cases of poisoning with iodoform through the vagina. The most important clinical cases of poisoning through the vagina, however, from the therapeutic and toxicological point of view are those with carbolic acid and bichloride of mercury.

Vetzel (14), von Herff (15), and others give accounts of poisonings with phenol through the vaginal route, some of which were fatal. A number of cases of black urine in female patients described by various authors must be undoubtedly attributed to phenol poisoning.

In respect to the absorption of mercuric chloride through the
vagina, even a larger number of cases is on record.   We will
not consider in this place poisoning following the use of mer-
curial douches in the post-partum state.   Such cases have been
described by Mabbott (16), Butscher (17), Wilhams (18), Kor-
nalewski (19), Bacaloglu (20), Edebohls (21), Le Clerc (22),
Legrand (23) and others: but they may be attributed in a large
measure to absorption through the puerperal uterus.   There
are, however, other cases of mercurial poisoning in which uterine
absorption must be considered as playing no rôle.   Collections
of such intoxications have been published by various authors
and may be found in a number of theses such as those by
Delaunay (24), Bonnet (25) and Garcia (26).   Witthaus (27)
collected one hundred and forty-three cases of mercurial poison-
ing with forty-eight fatalities, many of which occurred in the non-
puerperal state.   Sebillotee (28) described one hundred and forty-
eight such cases with thirty fatalities, and sporadic individual
cases of such poisoning have been reported by Gore (29), Wood,
Jr. (30), Fleischmann (31), Conaway (32), Millar (33), Tomel-
lini (34), Horn (35), and several others.   Beekman (36) de-
scribes a case of mercurial poisoning following a douche with
a dilute solution of bichloride of mercury, 1 : 2000, employed
to prevent conception.   Patek (37) reports a case of poison-
ing with bichloride following a vaginal douche in an unmarried
woman thirty-five years old.   Lankford (38) reported a re-
markable case of mercurial poisoning following the introduc-
tion of two antiseptic tablets into the vagina by a woman un-
acquainted with the manner of using vaginal douches.   Sharp
(39) reported a case of fatal poisoning in a little girl following
the insertion of a mercurial tablet into the vagina and Schil-
decker (40) described three other cases of death following the
introduction of mercurial tablets into the vagina, one in the
case of a girl and two in the case of young married women.
    The histories just cited are sufficient to emphasize the enor-
mous toxicological, hygienic and sociological importance of the
rôle played by the absorption of various poisons through the
vaginal route.   The gravity of the matter has heretofore been

entirely inadequately appreciated and for this reason the results of the present investigation are published.

CONCLUSIONS

1. It has been shown that a large number of drugs and poisons —alkaloids, inorganic salts, esters and antiseptics—can be and are easily absorbed through the vaginal wall.

2. Such absorption can be demonstrated experimentally by physiological and chemical means.

3. A review of the clinical and toxicological literature shows that posioning through the vagina, of a grave character, is not very rare.

4. The above-described experiments indicate, on the one hand, the possibility of administering drugs therapeutically for their constitutional effects, through the vaginal route; and on the other hand emphasize the great danger of the indiscriminate employment of various poisonous substances in the form of douches, tampons, "uterine wafers," etc.

The above investigation is intended as a war-time contribution tending to the prevention of ~~material~~ and infant *mater* mortality and the preservation of the race.

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LOCAL HEMOSTATIC PROPERTIES OF COTARNINE AND SOME OTHER AGENTS[1]

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Object of investigation. Claims have been made by K. Abel (1), Kehrer (2), Munk (3), Hulish (4) and Offergeld (5) that cotarnine hydrochloride (stypticin), cotarnine phthalate (styptol) and sodium phthalate act as efficient styptics or hemostatics both clinically and under experimental conditions. Their claims refer chiefly to the use of these drugs in uterine hemorrhages for which a certain degree of specificity is c'aimed (K. Abel), and also in connection with tooth extraction and hemophilia. Experimentally these claims have not been supported by the work of Laidlaw (6) on the coagulation of blood and blood pressure, and by Pilcher and Sollmann (7) on the vasomotor center (by direct stimulation). The literature of the manufacturers contains claims which appear exaggerated, and the experimental method used by Mohr and described by the gynecologist, K. Abel, is new and untried. It seemed worth while, therefore, to reinvestigate this class of preparations, particularly the salts of cotarnine, with the following objects in view:

1. Reliability of the method of K. Abel.

2. Repetition of the experiments of K. Abel.

3. Applications of other methods, which might suit the conditions and answer the claims that are made.

4. Comparison of the cotarnine salts with some well known and other hemostatics.

These will be discussed alone or together as appears suitable.

K. Abel's method. The description of this method by **K.** Abel (1) is as follows:

If the skin on the ball of the foot (of a cat) be cut sufficiently deeply to expose the papillary layer, hemorrhage will ensue (which is spontaneously checked with difficulty, and which will recur merely on rubbing). If such a wound be touched with styptol or phthalate of ammonium the hemorrhage ceases after a short time and the wound assumes a pale red aspect, and it may be seen that the blood in the capillaries has coagulated.

The following description by Kehrer (2), while somewhat different, is essentially the same as K. Abel's and is cited because sodium phthalate was used in my experiments instead of the ammonium salt which apparently is unessential and possesses no specific properties:

If a piece of skin is excised from the ball of the foot of a cat so that the papillary body is exposed, a hemorrhage will follow which only rarely ceases spontaneously and which can be increased by friction. If the solution of a neutral cotarnine salt or a neutral phthalate is applied to this wound, the hemorrhage will soon cease and the wound will appear pale red. The blood will clot in the capillaries themselves. If instead of a phthalate another salt (NH_4Cl or ordinary salt) is employed the hemorrhage will not be checked, but on the contrary will become worse. The local hemostatic action of cotarnine phthalate (styptol) must therefore be regarded as specific for the cotarnine as well as the phthalic acid.

Limitations of the method. In my experiments the technique as described by K. Abel was carried out as closely and carefully as possible. On the whole it was impossible to make satisfactory preparations of the cat's pad. In order to secure bleeding for a suitable period of time it was necessary to incise the pad. Removal of the skin of the pad alone produced practically no bleeding. The procedure, therefore, of removal of the skin together with the deep papillary layer was adopted. This secured a free bleeding surface which continued to bleed for variable periods of time, though never indefinitely.

The bleeding appears to be of two kinds: (1) a capillary

oozing from the papillary layer proper, and (2) venous from the venules underlying the skin and surrounding the edge of the wound. The bleeding from the venules is faster and more voluminous than from the papillae where it is slow and scanty. As a rule the veins continued to bleed irrespective of the application of drugs, pressure, or any other procedure during a certain interval, although after a sufficiently long interval the bleeding also stopped spontaneously. The oozing from the papillae could be diminished and stopped by pressure and certain drugs, but this was always difficult to judge on account of the contamination of the field with blood from the larger vessels, chiefly veins. Attempts were made to exclude the venous bleeding by continual mopping with pledgets of cotton, but the disturbance is so great and the success so limited that this practice was abandoned except with the larger veins.

Another limitation of the method is that continuous venous oozing removes the drug by "washing away." Repeated applications for this reason are necessary to insure continual contact of the drug with the wound.

The selection of a suitable control also proved difficult. The use of two pads from two different feet, one of which could serve as a control, and the other for treatment with drugs, proved unsuccessful because of the differences in the hemorrhages even with the most painstaking attempts to make the wounds alike in all respects. After some experimentation, a standard was found which could serve more or less efficiently for comparing the effects of other drugs. Applications of the drug to be tested were made to the wound and after a sufficiently long interval, epinephrin (1: 1000), which I took as the standard, was applied. If the bleedng did not stop as soon as after the application of epinephrin, it was concluded that the drug was less efficient. Results obtained in this way, and by other methods are described in the protocols.

From all that has been said, it is apparent that the cat's pad method of testing hemostatics as used by Mohr and described by K. Abel is crude and far from ideal, even with the use of the slight modification that I have introduced. The results are

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presented for whatever they are worth. Briefly, it may be stated that the results obtained were entirely negative for stypticin, styptol and sodium phthalate, but that epinephrin (1: 1000) can stop bleeding temporarily and more or less effi-

TABLE 1

Effect of hemostatics on bleeding from cat's pad*

TREATMENT	HEMMORRHAGE			NUMBER OF APPLICATIONS	REMARKS
	Stoppage lasted	Stopped at end of	Continued at end of		
	minutes	*minutes*	*minutes*		
Untreated	83				Superficial wound
	8				
Epinephrin 1: 1000	1	19		2 (drops)	
	3	5		Irrigation	
	2	44		Several irrigations	
	3	$7\frac{1}{2}$		Several irrigations	
Stypticin 10 per cent			$21\frac{1}{2}$	7	
			18	3	
			10	2	
Stypticin (dry) after epinephrin			7 (oozing)		
Styptol 10 per cent			6		Several irrigations
			19	7	
Styptol (dry)			10	2	
Styptol (dry) after epinephrin			7		
Sodium phthalate 10 per cent			4	Continual bath	
			10	3	
Tannin 1 per cent			16	4	
Tannin 10 per cent			$11\frac{1}{2}$	Several	
Tannin (dry)			10		
Hydrastinin 10 per cent			5	2	

* The wound was about 1 cm. by 1 cm.

ciently. In all, experiments on eight cats' paws were performed, in some cases the pads having been treated repeatedly. The discussion of the results follows.

Bleeding from the untreated cat's pad. The pad was incised

according to the procedure described above, and noting the time interval during which spontaneous stoppage of bleeding took place. The blood was mopped from time to time with a pledget of cotton. The following results were obtained: 8 minutes, $13\frac{1}{2}$ minutes, and 1 hour and 23 minutes, respectively. This shows the extreme variability of time within which cessation of bleeding can occur spontaneously, and obviously is a serious obstacle to judgment concerning the effects of hemostatics without some suitable standard.

Comparative effects of cotarnine salts and other agents on bleeding from the cat's pad. In the first series of experiments, the results of which are presented in table 1, various drugs, in solution and dry form, were applied directly to the wound by irrigation and dusting, respectively. It is seen that the application of epinephrin (1:1000) stopped hemorrhage in about 3 minutes and the stoppage lasted from 5 to 44 minutes. Almost immediately after the application of epinephrin, the wound appeared paler and gradually the oozing of blood stopped, though variably owing to contamination with venous hemorrhage. On the other hand, oozing continued uninterruptedly and unchanged at the end of from 4 to about 22 minutes (average 10 minutes) after the application of stypticin, styptol, sodium phthalate and tannin either in solution or dry form, and in spite of repeated applications. In fact, following the application of styptol and stypticin the hemorrhage appeared to be increased somewhat. Objection might be raised against the method of application, namely, that sufficient drug is not retained in contact with the wound on account of the continuous oozing from the venules which removes it by "washing away" and also dilutes it.

In the following series, therefore, the wounds were always mopped first, then irrigated with the solution and, in addition, a pledget of cotton placed over this was maintained saturated with the solution. When the powdered drug was used, this was reapplied from time to time and held in place by a pledget of cotton. The results of the experiments have been summarized in table 2, and are, in general, confirmatory of those in table 1.

Epinephrin (1 : 1000) partially stopped the hemorrhage in 2 to 3 minutes and practically completely in 5 to 12 minutes after

TABLE 2

Effect of hemostatics on bleeding from cat's pad

TREATMENT	HEMORRHAGE			MODE OF APPLICATION
	Stopped at end of	Stop-page lasted	Con-tinued at end of	
	minutes	*minutes*	*minutes*	
Epinephrin 1: 1000	2 (Partial)			Pledget of cotton
	12 (Complete)	20		Pledget of cotton
	2 (Partial)			Pledget of cotton
	7 (Complete)			Pledget of cotton
	3	20		Pledget of cotton
	4 (Partial)			Pledget of cotton
	12 (Complete)			Pledget of cotton
	3 (Partial)			Pledget of cotton
	8 (Complete)			Pledget of cotton
	3			Pledget of cotton
	3 (Partial)			Pledget of cotton
	5 (Complete)	16		Pledget of cotton
	5 (Complete)	13		Pledget of cotton
	3 (Partial)			Pledget of cotton
	5 (Complete)	12		Pledget of cotton
	6	11		Pledget of cotton
Stypticin 10 per cent			22	Pledget of cotton
			38	Pledget of cotton
Stypticin (dry)			31	Pledget of cotton
			42	Pledget of cotton
Styptol 10 per cent			22	Pledget of cotton
			36	Pledget of cotton
Styptol (dry)			29	Pledget of cotton
			40	Pledget of cotton
Sodium phthalate 10 per cent			23	Pledget of cotton
Kephalin* 5 per cent	(Slowed) 44			
	22 (Complete)			
	44 (Complete)			
	22 (Complete)			

* Armour's product, 5 per cent emulsion, in saline, was used.

application.   The stoppage lasted from 11 to 20 minutes.   Powdered epinephrin tablets (containing 1 mgm.) of epinephrin

appeared to be less efficient, for it is undoubtedly necessary for the epinephrin to be in solution before it can act.   Hemorrhage continued freely at the end of 22 to 42 minutes after the application of styptol, stypticin and sodium phthalate.   In fact, styptol seemed to increase the hemorrhage.   Kephalin slowed the oozing in 22 to 44 minutes after application, but in no case was there complete stoppage.

It may be concluded that styptol, stypticin and sodium phthalate (in strong solution or dry form) are inefficient as hemostatics for superficial wounds of the cat's pad as compared with a much weaker solution of epinephrin (1:1000), which is promptly effective though the effects are temporary and also somewhat variable.   The experiments with tannin (strong solution and dry form), hydrastinine and kephalin are too incomplete to permit the drawing of definite conclusions, but it appears that their hemostatic efficiency under the conditions they were used here is comparatively limited.

Experiments on the cock's comb. Applications of the various hemostatics were made to (1) superficially denuded areas (about 0.5 to 1 cm. by 0.5 to 1 cm.) of the comb, and (2) to wounds made by section across the tip of the comb.   The hemorrhage from the denuded areas was slow and occurring at discrete points in the white fibrous portion of the organ.   More bleeding took place from the edges of the wound.   The hemorrhage across the tip was freer and more voluminous, resembling the venous type.   The bleeding from this did not stop for a very long time, while in the untreated denuded areas the bleeding stopped of its own accord in 1 hour.   The following results (table 3) were obtained with the various drugs.

Epinephrin (1:1000) stopped the bleeding in 2 minutes and this lasted for 6 to 10 minutes.   Powdered epinephrin tablets stopped it in 3 minutes and this lasted 35 minutes.   On the other hand, hemorrhage did not stop at the end of 33 to 74 minutes after repeated applications of stypticin 10 per cent, styptol 10 per cent, sodium phthalate 10 per cent, glycerite of tannic acid (U. S. P.) and powdered stypticin.   In fact, after stypticin and styptol the oozing seemed to be increased in the same way

as in the experiments on cats' pads. The results, in general, are confirmative of those described in the previous section.

It may be concluded that the local application of epinephrin

TABLE 3

Effect of hemostatics on cock's comb*

TREATMENT	Stopped at end of	Stoppage lasted	Unchanged at end of	NUMBER OF APPLICATIONS	REMARKS
	HEMORRHAGE				
	minutes	*minutes*	*minutes*		
Untreated	60				
Epinephrin 1:1000	2	10	About 15; stronger at 30	3	
	2	6	33	1 cc. irrigation Continuous to wound across comb	
Epinephrin (dry) (1 mgm. tablet)	3.	35			
Stypticin 10 per cent			74	Irrigation and saturated cotton pledget for 60 minutes to wound	Bleeding increased
Stypticin (dry)			37	Repeated dusting on wound	Bleeding increased
Styptol 10 per cent			74	Irrigation and saturated cotton pledget for 60 minutes to wound	Bleeding increased
Sodium phthalate 10 per cent			37	Saturated pledget of cotton on wound	
Glycerite of tannic acid (U.S.P.)			35	Seven to wound across comb	

* The results from both superficial wounds and across the comb are embodied in the table.

promptly and effectively though temporarily stops bleeding from superficial wounds of the cock's comb, while stypticin, styptol and sodium phthalate are ineffective.

Effects on liver and spleen. (1) Excised organs. Freshly excised organs of the cat were used. The results are presented in table 4. Direct application of a powdered epinephrin tablet over an area of about 0.5 cm. by 0.5 cm. to the liver and spleen caused local blanching in 2 minutes and this lasted for about 5 minutes. At the end of this time the whitening effect disappeared, the site of application again resuming a dusky brown appearance, and slight oozing of blood continued. On the other hand, applications of powdered styptol and stypticin produced no change in the appearance of both the liver and the spleen, except for the brownish or straw color stain left after the use of styptol, this being the natural color of its solution. This holds true for all the experiments thus far and subsequently to be described.

(2) Intact liver. The application of 1 : 1000 epinephrin solution to an abrazed area (about 0.5 cm. by 0.5 cm.) had no effect when the oozing was rapid and voluminous. When the oozing was slow and not so voluminous, stoppage was produced almost immediately after application, and there was blanching of about the same kind and extent as in the excised organ. The following drugs, when applied in the same way as epinephrin, produced no visible effects on the bleeding; powdered styptol and stypticin, 10 per cent styptol and stypticin, 5 per cent kephalin, glycerite of tannic acid (U. S. P.) and lung extract (1 cc. of which was equivalent to 1 gram of the moist freshly excised organ of the dog).[2]

Uterus. The uterus of the guinea-pig does not appear to be a satisfactory test object. The non-pregnant organ does not bleed sufficiently, bleeding being impeded by the contraction of the cut muscles, and in the pregnant organ, the observations are impaired by movement of the fetus and bulging of amnion, which because of the pressure produced tend to stop bleeding naturally.

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[2] Fischl (Arch. Kindhkl., 1916, lxv, 188) has made the claim that lung extract is an excellent local hemostatic, the effects being so striking that a patent has been applied for. The strength of Fischl's extract is unknown to me. Fischl's claim would not gain support from a prior claim of Gutmann's (Compt. rend. soc. biol., 1914, lxxvi, 349), namely, that lung extract diminishes the coagulability of blood.

TABLE 4

Effect of hemostatics on viscera

TREATMENT	HEMORRHAGE			REMARKS
	Stopped at end of	Stop-page lasted	Continued at end of	
Intact liver of cat				
	minutes	*min-utes*	*minutes*	
Epinephrin 1 : 1000 {	2None Immediate			Blanching Oozing rapid Oozing slow
Epinephrin (dry)				Oozing slow
Styptol 10 per cent {			10 15	No blanching Oozing increased
Styptol (dry)			13	
Stypticin 10 per cent			Continuous	
Stypticin (dry)			14	
Sodium phthalate 10 per cent			10	No blanching
Kephalin 5 per cent			Continuous	
Glycerite of tannic acid (U. S. P.)			Continuous	
Intact tongue of cat				
Epinephrin 1 : 1000			5	No blanching
Styptol 10 per cent			5	No blanching
Freshly excised liver of cat				
Epinephrin (dry) {	Blanching only 2	4	Blanching only	
Styptol (dry)			No change during the afternoon	
Stypticin (dry)			No change during the afternoon	
Freshly excised spleen of cat				
Epinephrin (dry)	2	5		
Styptol (dry)			No change during the afternoon	
Stypticin (dry)			No change during the afternoon	

TABLE 4—Continued

TREATMENT	HEMORRHAGE			REMARKS
	Stopped at end of	Stoppage lasted	Continued at end of	

Intact non-pregnant uterus of guinea-pig

	minutes	minutes	minutes	
Epinephrin (dry)	1	$7\frac{1}{2}$		Blanching only at edge of wound
Epinephrin (1:1000)	1	6		Wound pale
Stypticin (dry)			Continuous bleeding end of eighteen minutes	No blanching; bleeding increased
Stypticin, 10 per cent			Continuous bleeding end of eighteen minutes	No blanching; bleeding increased

Intact pregnant uterus of guinea-pig

Epinephrin (dry)	8	8		Blanching only at edges of wound
Epinephrin (1:1000)	1	10		Wound blanched
Stypticin (dry)			Bleeding continued end of 17 minutes	When embryo moves and bulges amnion into wound, bleeding stops; no blanching
Stypticin 10 per cent			Bleeding continuous	No blanching; oozing increased

Intact pregnant uterus of guinea-pig. (No wounds; direct application of drugs to organ)

Epinephrin (dry)	Blanching in 1 minute; disappeared at end of 9 minutes after application
Stypticin (dry)	In 2 minutes, uterus became redder; 4 minutes, later organ very red, organ injected with blood, redness spreading beyond place of application of stypticin; in 6 minutes, organ is "angry-looking," very red, vessels are tortuous and stand out prominently. At end of 20 minutes, after application, redness lessened, vessels were less tortuous. At end of 30 minutes organ appears about same as before application of stypticin.

However, a few observations were made on the non-pregnant and pregnant organ of the guinea-pig. The data from these are presented in table 4.

Pregnant uterus. When a pulverized epinephrin tablet containing 1 mgm. of epinephrin was placed on the organ, blanching occurred in 1 minute, and this lasted for 9 minutes. On the other hand, when dry stypticin was applied, the organ appeared more turgid with blood and the vessels were more tortuous In other words, a rather marked hyperemia occurred which spread beyond the place of application of the drug, and this lasted for about half an hour.

The application of dry epinephrin to a bleeding abrasion resulted in reduction of the hemorrhage in 4 minutes, and complete stoppage took place in 8 minutes. After the application of dry stypticin to a similar wound, the hemorrhage was markedly increased and continued so at the end of 17 minutes. When the fetus moved, and the amnion bulged into the wound, the bleeding stopped momentarily, but resumed again when the pressure was removed.

Epinephrin 1:1000 solution, stopped bleeding in 1 minute and this lasted for about 10 minutes, while the application of 10 per cent stypticin caused the bleeding to increase.

Non-pregnant uterus. The results were similar to those with the pregnant organ. Epinephrin, in dry form (1 mgm. tablet) and solution (1:1000), stopped bleeding in 1 minute and this lasted for about 7 minutes. Stypticin in dry form and solution (10 per cent) caused an increase in the bleeding.

The results obtained with the uterus are, therefore, confirmative of those obtained with the other viscera. Uterine vessels were perfused, and this will be referred to presently.

Peripheral vasoconstriction in the rabbit's ear. There is no reason to believe that epinephrin, as used in the experiments described above, acts as a hemostatic in any other way then by local vasoconstriction. Whether it involves both the capillaries and arterioles or only the one or the other is immaterial here. The fact that it was effective is indicative of its absorption. It may be that the cotarnine salts (stypticin and

styptol) as well as the other agents can also act by vasocon-
striction, and the objection might be raised that they did not
exert any such action here because they were either not ab-
sorbed or only incompletely so and thereby prevented from
exerting their action on the vessels. Injections of the various
agents, therefore, were made into the base of the rabbit's ear
in the vicinity and around a large vessel according to the method
recently described by Meltzer and Auer (8).

With epinephrin (1:1000), blanching begins to take place in
less than 1 minute and practically the entire ear is white at the
end of 1 to 3 minutes, and cold to the touch. This persists for
several hours, about 7 to 24. The effects are very striking and
were repeatedly obtained. This is confirmative of Meltzer and
Auer.

In the first series (part A of table 5) of experiments, one ear
was used for the epinephrin control and the other ear was
treated with the drug to be tested. Following the injection
of styptol (10 per cent) and stypticin (10 per cent), the ears
remained warm and no blanching was visible at the end of 17
to 64 minutes. The epinephrin controls in each case responded
in the usual manner. The styptol and stypticin ears remained
unchanged the entire afternoon (4 hours).

In another series of experiments (part B of table 5), the ears
were first treated with the different agents and, after a given
lapse of time, epinephrin was injected into the same place
where the drug to be tested was injected. The results obtained
were as follows:

Styptol (10 per cent): No blanching was observed and the
ears remained warm at the end of 1 hour and 15 minutes and 1
hour and 17 minutes, respectively, after injection. Injection of
epinephrin (1:1000) into the same ears, and in the same place
where the styptol was injected, produced blanching and cooling
within 1 minute after injection.

Stypticin (10 per cent): No blanching was observed and the
ears remained warm at the end of 1 hour and 5 minutes and 1
hour and 9 minutes, respectively, after injection. At the end
of this time, injection of 1:1000 epinephrin into the stypticin

TABLE 5

Effect of hemostatics on vessels in rabbit's ear

DRUG INJECTED	CONDITION OF EAR (PALING OR WHITE DENOTES VAOSCONSTRICTION)	TEMPERA- TURE OF EAR	REMARKS
	Part A		
Epinephrin (1 : 1000)	Begins to pale in less than $\frac{1}{2}$ minute	Cool	Nicotinized rabbit
	Begins to pale in less than 1 minute; completely white in 3 minutes	Cold	Normal rabbit
	Beginning paling in less than 1 minute; completely white in 3 minutes	Cold	Normal rabbit
Stypticin 10 per cent	No paling end of 27 minutes	Slightly cool	Nicotinized rabbit
	No paling end of 64 minutes	Warm	Normal rabbit
Styptol 10 per cent	No paling end of 28 minutes	Warm	Nicotinized rabbit
	No paling end of 17 minutes	Warm	Normal rabbit.
	Part B		
Styptol 10 per cent	Ear unchanged; no paling end of 1 hour and 15 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent in site of styptol injection	Beginning paling within 3 seconds; ear completely white in 1 minute	Cold	"
Styptol 10 per cent	Ear unchanged; no paling end of 1 hour and 17 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent in site of styptol injection	Beginning paling in less than $\frac{1}{2}$ minute; completely white in 1 minute	Cold	"
Stypticin 10 per cent	Ear unchanged; no paling end of 1 hour and 5 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent in site of stypticin injection	Paling in 2 seconds; ear completely white in 2 minutes	Cold	"
Stypticin 10 per cent	Ear unchanged; no paling end of 1 hour and 9 minutes	Warm	Normal rabbit

TABLE 5—Continued

DRUG INJECTED	CONDITION OF EAR (PALING OR WHITE DENOTES VASOCONSTRICTION)	TEMPERA-TURE OF EAR	REMARKS
Epinephrin 0.1 per cent in site of styptol injection	Ear completely white in 1 minute	Cold	Normal rabbit
Lung extract	Ear unchanged; no paling end of 49 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent in site of lung extract injection	Ear white in 2 minutes	Cold	"
Lung extract	Ear unchanged; no paling end of 52 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent in site of lung extract injection	Ear white in 1 minute	Cold	"
Kephalin 5 per cent	Ear unchanged; no paling in 22 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent in site of Kephalin injection	Ear white in 1 minute	Cold	"
Hydrastinin 10 per cent	Paling around vessel injected in 22 minutes	Warm	Normal rabbit
Epinephrin 0.1 per cent in site of Hydrastinin injection	Ear white in 1 minute	Cold	"

areas of the same ears was made with the result that marked blanching and a sensation of coolness to the touch throughout the ear occurred within 1 minute.

Other agents: Lung extract and kephalin (5 per cent) produced no effects whatsoever after 49 and 52 and 22 minutes, respectively, whereas injection of 1: 1000 epinephrin in the previously injected areas of the same ears gave the usual responses of blanching and cooling within about 1 minute. Following the injection of hydrastinine (10 per cent) a small area of paleness surrounded the blood vessel around which the injection was made in 22 minutes; the remainder of the ear and the portion supplied by the vessel exhibited no unusual changes. Injection of 1: 1000 epinephrin here again promptly produced the usual responses within one minute.

It may be concluded that epinephrin in weak solution (1: 1000)

acts as a prompt and powerful vasoconstrictor when injected into the base of the rabbit's ear. Strong solutions (10 per cent) of stypticin and styptol when administered under the same conditions as epinephrin produce no such demonstrable vascular effects. The injection of epinephrin after the injection of styptol or stypticin into the same ear promptly produces the usual vaso-
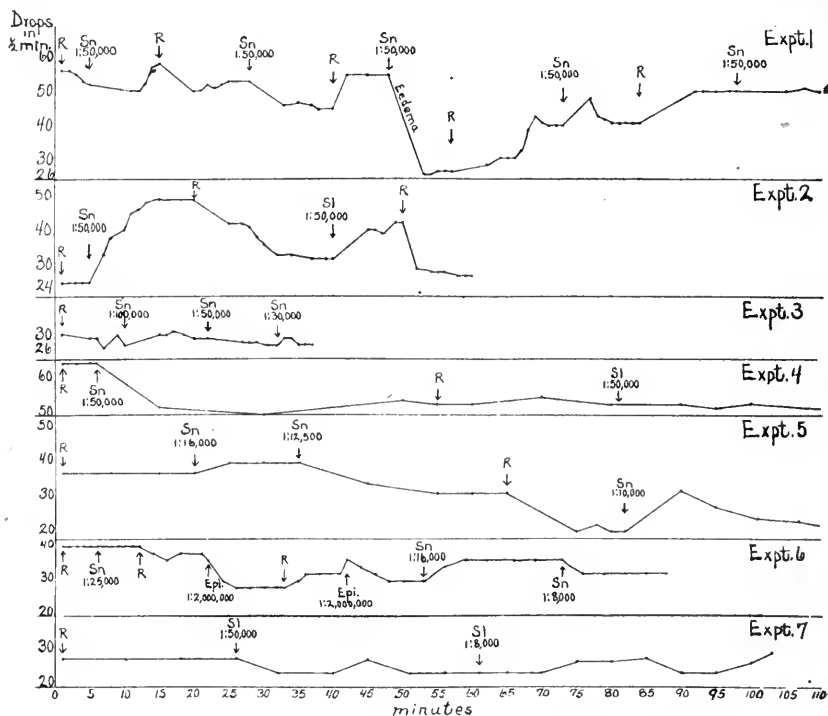
FIG. 1. EFFECT OF COTARNINE SALTS ON FROG'S VESSELS

The abbreviations have meanings as follows: *R.*, frog's Ringer solution; *Sn.*, stypticin (cotarnine hydrochloride); *Sl.*, styptol (cotarnine phthalate); *Epi.*, epinephrin. All drugs were dissolved in frog's Ringer solution.

constrictor effects as in the untreated ear, showing that the blood vessels are responsive and not affected by previous treatment with strong solutions of stypticin and styptol.

Perfusion of frog's vessels. This was done in the usual way by the Läwen-Trendelenburg method. The results obtained are presented in figure 1.

Stypticin. Sixteen applications of stypticin in concentrations varying from 1: 100,000 (0.001 per cent) to 1: 10,000 (0.01 per cent) were made in six different experiments. The results are somewhat variable. The majority (50 per cent) indicate definite relaxation of the vessels, and of the remaining about 38 per cent showed varying degrees of constriction. About 12 per cent of all the applications showed no change. This variability may be accounted for by the presence of oedema in some of the experiments as a complicating factor.
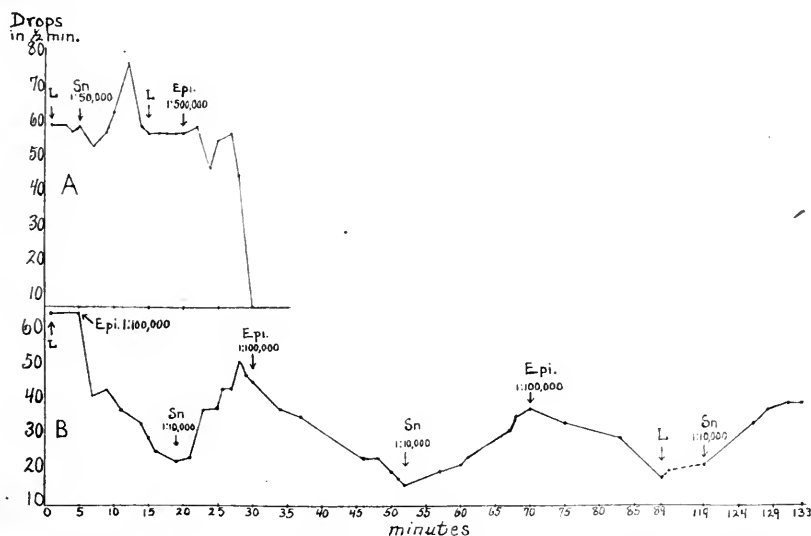
FIG. 2. EFFECT OF COTARNINE HYDROCHLORIDE (STYPTICIN) AND EPINEPHRIN ON UTERINE VESSELS

The abbreviations have meanings as follows: *Sn.*, stypticin; *Epi.*, epinephrin; *L.*, Locke's solution. The drugs were dissolved in Locke's solution.

Styptol. The majority of four observations made with styptol in concentrations ranging from 1: 50,000 (0.002 per cent) to 1: 8000 (0.0125 per cent) indicate a definite vascular dilatation.

In this connection, it may be added that similar effects were observed on the perfused frog's heart (Straub method). Of the two salts, styptol (cotarnine phthalate) was more depressing than stypticin (cotarnine hydrochloride). It is obvious that

these results would have only an indirect bearing on hemostasis. In some experiments, stypticin produced a moderate increase in pulse rate with depression of or no change in amplitude and tonus.

From all this it seems permissible to conclude that the effects of stypticin and styptol on the peripheral vessels of the frog are unfavorable to the claims made for their local hemostatic properties.

Perfusion of uterine vessels. The cornua of a rabbit's uterus were perfused (at room temperature) separately according to the Läwen-Trendelenburg technique. The results are presented in figure 2. From curve A, which represents perfusion of the vessels of the right cornuum, it is seen that stypticin produced dilatation, while epinephrin (used as a control) caused constriction. The results in curve B, which represents perfusion of the left cornuum, show that stypticin relaxes vessels previously constricted by epinephrin. The results are similar to those obtained on frog's vessels, and confirm also the observations made with direct application of stypticin to the uterus.

It is justifiable to conclude that the effects of stypticin (cotarnine hydrochloride) on uterine vessels are not conducive to hemostatic efficiency. In fact quite the contrary would be expected, that is, an increase in hemorrhage.

SUMMARY

1. Epinephrin (1:1000 and powdered tablets containing 1 mgm.) acts as an efficient hemostatic for small and superficial wounds. The effects are temporary and somewhat variable. It seems to be ineffective for stopping venous hemorrhage.

2. Stypticin and styptol (10 per cent solutions and dry powders) are inefficient as local hemostatics, even after long periods of and repeated application. In fact, hemorrhage tends to be somewhat aggravated after the application of these agents, and styptol is irritating.

3. Epinephrin (1:1000) is a prompt and effective peripheral vasoconstrictor in the rabbit's ear, while styptol and stypticin (10 per cent solutions) are inactive and inefficient. Styptol and

stypticin produce relaxation of frog's vessels, and stypticin of uterine vessels.

4. The following agents do not appear to act either as local hemostatics or peripheral vasoconstrictors; sodium phthalate 10 per cent, lung extract 100 per cent, kephalin 5 per cent and hydrastinine hydrochloride 10 per cent. With the exception of the phthalate, however, final conclusions are reserved until experiments with these drugs have been extended.

CONCLUSIONS

The evidences obtained, it is believed, justify the following conclusions regarding the original objects of the investigation.

1. The original method described by K. Abel is at best a crude method, rendering positive deductions from results obtained with it somewhat hazardous unless fortified by other methods of observation.

2. The claims made by K. Abel that stypticin and styptol act as hemostatics on the incised pad of the cat's paw have not been confirmed.

3. Other methods that were used for testing out the alleged hemostatic properties of stypticin and styptol also failed to demonstrate any such effects.

4. As compared with epinephrin, the salts of cotarnine (styptol and stypticin) and sodium phthalate are certainly inferior hemostatics, and probably entirely inactive in this respect. This conclusion is fully confirmed by results which I have since obtained with a quantitative method for the study of local hemorrhage, and shortly to be reported.

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